

nature

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What can scientists contribute to arms control?

ARMS control and disarmament have had an unexpectedly active summer. Not that anybody has actually done anything about controlling arms or putting them out of harm's way, but there has been more public talk than for a long time. *Daedalus* has followed up, fifteen years on, its immensely valuable Fall 1960 issue on arms control with a Summer 1975 issue devoted to "Arms, Defense Policy, and Arms Control." The World Federation of Scientific Workers (WFSW) has recently held a major symposium ("The role of scientists and of their organisations in the struggle for disarmament") in Moscow. And the thirtieth anniversary of Hiroshima has occasioned some thought-provoking events, particularly (on BBC Television) a remarkable interview with Professor Philip Morrison who gave a lucid description of the way things were and the way people thought in those days.

There has been a nice contrast about it all; the harsh realities of Morrison, the populist aspirations in Moscow, the purring sophistications of Cambridge, Massachusetts. But have science and the scientist any future in the field of arms control? The WFSW has no doubt; "scientific workers can provide the ammunition to make the force of public opinion [for ending the arms race] overwhelming". *Daedalus* is less sure: "... arms control and disarmament now appear to be politically more complex than they did in 1960, while the technical aspects (of verification and the like) appear somewhat less complicated and perhaps therefore less consequential", writes Franklin Long.

The similarities between atomic weapons and arms control are striking. There was a time when certain key scientists could make direct approaches to politicians and be given a fair hearing for their views on whether and how a bomb should be built. But once government had been told how to do it, a large machine took over and nuclear weapons became no longer a crusade but an industry. So it was with arms control. There was a time when scientists had governments' ears for their views on verification; a black box here, Mr President, a satellite there, two dozen seismometers here. There were heady times, as many will testify, when it really looked as if significant nuclear limitations might have been negotiated on the basis of straightforward technical advice. No longer; the scientists gave what they knew, the machine of state absorbed it and moved on. Even arms control became less of a crusade and more of an industry.

It also became infinitely more complex, of course.

Whereas it used to be possible to talk of banning this or that, it is now necessary to deal in packages; restricting (not banning) this or that, swapping information on space medicine and wheat yields, exchanging works of art, making visas easier to get and so on. The scientist's role as an input of scientific information is a pretty minor one and at least in the nuclear field is likely to remain so.

It has also to be remembered that for every one scientist who sincerely deems it his or her responsibility to speak out in the few forums available (in the UK practically none at all, since the community of intellectuals is otherwise preoccupied) there is at least one other scientist who deems it his or her responsibility to work diligently to keep the nation's defences intact. Thus talk of Hippocratic oaths for scientists to eschew work on projects which might injure or kill humans or calls for scientists to measure up to their social responsibilities might, if taken seriously, lead to holier-than-thou witch-hunts in the scientific community.

If one cannot be optimistic that scientists *per se* have a major role to play in arms control discussions in general, there are perhaps two areas in which they can be particularly effective. The first includes weapons of the twenty-first century (perhaps, in particular, environmental weapons). Since none of these are at the research and development stage yet, there is likely to be a much greater agreement that they should be eschewed. Scientists are notoriously conservative in predicting the future of their subject, but surely if a major international effort were to be devoted to identifying danger areas, it is not inconceivable that an international consensus could be reached on control measures before rather than after the threat was in existence. Second, scientists could make a ruthless examination of the research and development aspects of every proposed arms control measure and ensure the appropriate publicity.

- Research and development is the mainspring of military improvement; beware of any agreement that leaves the laboratories intact.

- The Partial Test Ban Treaty was even used as a justification for the United States to increase its nuclear research and development activities.

- SALT has been a positive encouragement to qualitative improvement of missiles.

Nobody knows this as well as the informed scientist, and it may on occasions even be necessary for apparently benign measures to be opposed.





The East is read

To explore one important facet of the visible tip of the iceberg of Chinese science, S. Dedijer and B. Billgren ask: what was the effect of the Cultural Revolution on *Scientia Sinica*, the principal journal of the Chinese Academy of Sciences? How and to what extent has politics caused it to depart from the international scientific tradition?

AS the chief Academy journal, *Scientia Sinica* occupies a unique position both inside and outside China. Although, as a result of the Cultural Revolution, the journal ceased publication for seven years in 1966, it is in China the standard bearer of science and the most prestigious channel for scientific communication. Abroad it is the official broker of knowledge on the state of China's science and stakes out China's claims amongst world scientific competition. Thus, focusing on this very visible yet little studied face of Chinese science, one learns not only about changes from 1965 to 1974, but gains an insight on the present state of the scientific community in China.

Our basic materials are the four volumes (XIV–XVII: 1965–1974) with 28 issues and 405 articles) of the foreign language edition of *Scient. sin.*, identical to the Chinese edition (we compared two 1973 issues of each). To this material we applied a number of simple qualitative and quantitative indicators for the international scientific tradition and its change by politics.

Tradition: (1) the journal as communication channel; (2) absence of politics in articles; (3) structure of the

journal; (4) structure of articles; (5) science norms; (6) style; (7) priority protection.

Changes by politics in: (1) editorial policy—including “mass science” and “serve the people”; (2) political content of articles; (3) authorship of articles; (4) research programme in journal; (5) language of articles; (6) institutional changes; (7) origin of references; (8) rapidity of publication.

An analysis of the 405 articles shows that all overt political statements in them can be classified under one of the following categories—

- Mao citation: “Chinese medicine is a great treasure which should be investigated and further developed for the welfare of mankind (quoted twice during 1973).

- Political statements: “Since the founding of New China and under the correct leadership of the Chinese Communist Party” in “Cure of Choriocarcinoma and Chorioadenoma Destruens Chemotherapy” (No. 2, 297; 1973).

- Sentences with overt marxist-maoist orientation: “The correct road of dialectical materialism can be only assured by going deeply into the essence of this problem” in “Crustal Structure and Crustal Movement” (No. 4, 520; 1973).

- Policy Statements: “We are still a developing country. Steps have just been taken to study the aforementioned significant theoretical problems of science” (such as pulsars, quasars and background cosmic radiation) in “Heliocentric Theory in China” (No. 3, 376; 1973).

The political content of articles is measured on a 0, 1, 2 scale: 0 for articles without a single overt political sentence, 1 for one or two such sentences, 2 for more than two.

At the beginning of the Cultural Revolution in 1965–66 there were strong hints in the literature that the publication of scientific journals was anti-revolutionary. The most revolutionary impact of the Revolution on *Scient. sin.* was that it ceased publication for almost seven years together with all the other journals, except *Chinese Medicine*, which continued until 1968 in a highly political form. With the renewed publication of the foreign and Chinese editions of *Scient. sin.* (and 19 other journals) in 1973, China returns not only to the 300-year-old international tradition but also to its pre-1949 tradition. For *Scient. sin.* is the new name given in 1955 to the Chinese Academy journal *Acta Scientia Sinica* begun in 1952, which in its turn succeeded *Science Record*, the main Academy journal in 1942–45 under Ch'ang Kai Shek's government. The editor of that, as well as of *Acta scient. sin.* from 1952 was the Chicago

trained physicist Y. H. Woo, vice-president of the Academy from 1949 until after the Cultural Revolution.

Exactly resembling the corresponding Western academy journals and its Chinese predecessors, *Scient. sin.* throughout its history up to issue No. 2 of 1974 consisted exclusively of articles communicating to the Chinese and the world scientific community the latest research results in “pure science, technology, agriculture and medicine with emphasis on basic theoretical research”. Several scanings of the 405 articles show that all those in 1965–66 and 74% of those in 1973–74 follow the Chinese and Western tradition: they contain not a single overt political sentence.

All the 405 scientific articles from 1965 to 1974, even those with political content 2, have like their Chinese predecessors the traditional article structure. The title is followed by the name of the author(s), the institution(s), “date received”, “abstract”, “introduction”, followed by the traditional sections “method”, “experimental results”, depending on the subject. All the articles in *Scient. sin.* close as elsewhere with sections “conclusion” or “discussion”, “acknowledgements” and “references”.

The fact that following the international tradition all the 1973–74 articles record the date the article arrived on the editor's desk is related—as are all other components of the standard article structure—to specific socio-cognitive components of the research process. This “date received” usage has at least two functions: it stakes a scientist's claim to priority in his field, and it measures the speed of publication. Encouragement of international competition in science and technology has been a constant policy of the Chinese leadership. Mao, Lin Piao, Liu Shao Chi, and Chou En Lai have continuously stressed that “the country will certainly catch up with and surpass advanced world science levels in the not distant future” (*Scient. sin.*, No. 2, 279; 1973). Of the 235 articles in 1965 only one was published within a month of receipt. This was the most significant Chinese contribution to world science since 1949; synthesis of the insulin molecule by a group of 21 authors (*Scient. sin.*, No. 11, 1965). The three articles on the subject in 1973 and the two in 1974 were also published more rapidly than the average waiting time of six months. The only two articles in 1973–74 published within two months of receipt had definite political overtones; both were results of collaboration between an American (of Chinese descent) and a Chinese scientist.

A detailed study of the style and content of articles in 1973–74, even the

most political, shows a consistent effort to be impersonal and objective in style. The authors express themselves with the usual professional researcher's methodological reservations and scepticism. They relate their ideas and results to those of others and to knowledge already confirmed. There is a continuous professional striving toward a scientific approach, results and conclusions. Hence all the articles—even the most political—follow at least three of Merton's "institutional imperatives" of professional ethics of science. The 300 of 405 1973-74 scientific articles with no political statements can be said to follow the fourth, the "disinterestedness" imperative; they contain no explicit statements on the social significance of the reported research either in "serve the people" language or any other.

Only two out of 93 articles in 1973-74 are directly related to the important goal of the Cultural Revolution—"science by the masses". One in 1973 is by a peasant on "Using Chairman Mao's Philosophical Thinking to Guide Scientific Experiment in Peanut Cultivation". The second in 1974 is by a "worker-instructor" on "A Mathematical Analysis of Involute Gear Periodic Errors". Both articles—one with a political content of 2, and the second with 0, follow closely the traditional article structure.

The political content of articles and of the journal are shown in Fig. 1. As seen, there was no politics in 1965-66 articles. During 1973, 56% (24 out of 43) had no political content compared with 86% (43 out of 50) in 1974. From 1973, issue No. 1, the political content of articles decreased continuously and in 1974 it amounted to one or two points per issue. These "political bits" are to be found with few exceptions in the introduction or conclusion sections, and many are repeated in several articles giving the impression of being insertions by the editors.

With No. 2, 1974, two sorts of purely political articles began to appear. The first, at the beginning of each issue are political editorials criticising Lin Piao, Confucius and "the theory of innate genius". The second, placed at the end of the issue are philosophical "Studies on History of Natural Sciences". Such articles (Fig. 1) take about 10% of the pages of the journal. Thus it seems that the political bias of purely scientific articles is being replaced by a more pronounced political editorial policy with the double goal of guaranteeing the political correctness of the journal and the ideological education of the readers.

Figure 2 shows some significant changes, probably politically induced, in the articles in 1973-74 as against

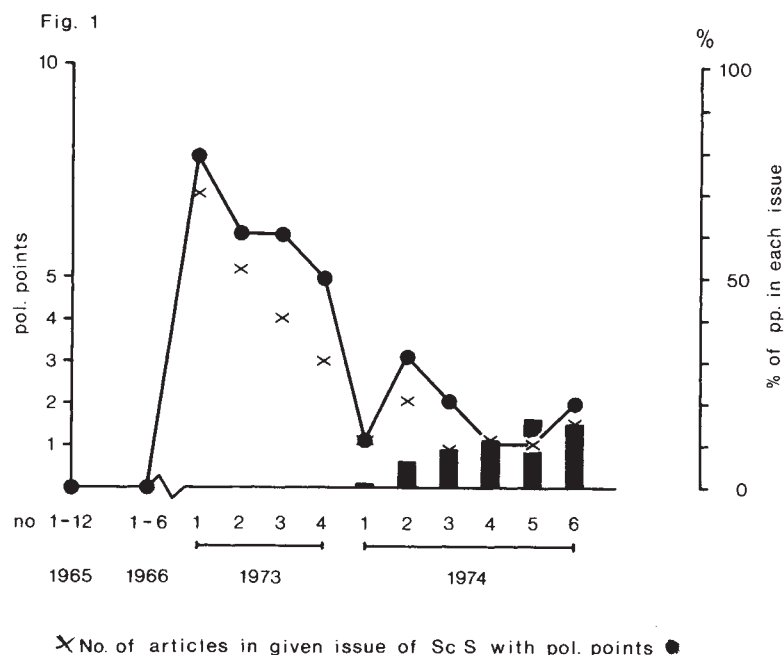
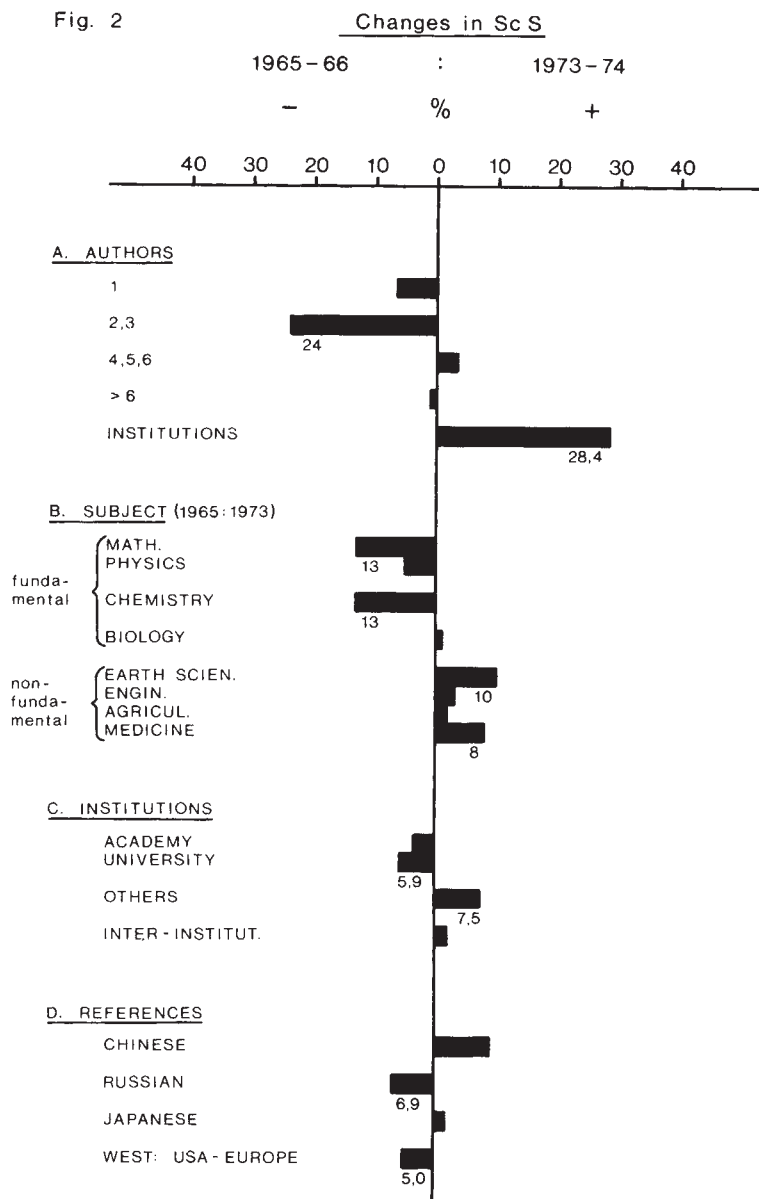


Fig. 2



1965–66. From *a* we note a significant decrease of articles by 1, 2 or 3 authors and an increase in collective authorship giving only the name of the institution(s). This may turn to be temporary for it makes difficult the communication among researchers working on the same subject. Figure 2*b* shows a shift between 1965 and 1973 from the theoretical work in fundamental to non-fundamental sciences. It seems that *c* shows no significant shift in the pattern of institutions performing the work except for the decrease of that in universities. As regards the national origin of references (a total of 3,533 in 1965–66 against 1,242 in 1973–74) we note in a 6.9% decrease of Russian and 5.9% of Western, the latter amounting to 60% of all 1973–74 references. In 1965–66 there were 22 articles in Russian and 9 in French compared with 1 and 2 in 1973–74.

Although our method is relatively simple, it seems to us that a more refined one would not lead to radically different results. We conclude, first, that our results from *Scientia Sinica* do not support such widespread general conclusions by visitors, as for example by A. Peyrefitte, namely, that in the Cultural Revolution "Scientists have been dealt a body blow. The functioning of research has been revolutionised to a degree which is hard to imagine, except, however, for national defence projects".

We find quite the contrary situation. Except for a very significant quantitative decline (312 articles and notes in 18 issues in 18 months of 1965–66 against 93 articles in 8 issues of 24 months of 1973–74) our results indicate that the goals of the Cultural Revolution are not reflected to any significant degree in the 1973–74 *Scientia Sinica*. All our evidence shows that the science China is most proud to show the world is still done entirely by professional scientists, who for their ideas and research problems still turn, as in the rest of the world to national and international science itself and not to the masses. This points to the existence of a scientific community in China which functions by the same traditions, norms and usages as in USSR, USA and Europe. We also note that in the period studied, the Chinese have not made any new innovations in this tradition which are visible in *Scientia Sinica* except for the radical increase of "collective authorship" of dubious value. Consequently we conclude that—with the exceptions noted—the *Scientia Sinica*, has followed in 1973–1974, as throughout its history, the international tradition of science, according to chairman Mao's directive "Make the past serve the present and foreign things serve China".

Barefoot in the field

Though a thorough study of the barefoot doctor system has yet to be carried out, there is no doubt that it represents the first successful, large-scale attempt at the delivery of total health care in a developing country cut off from Western civilisation. Alexander Dorozynski discusses the system by which, in the space of a few years, China has managed single-handedly both to overcome chronic malnutrition and to bring under control most of the epidemic diseases which have plagued it in the past.

THE really significant developments in health care delivery in China have taken place during the past 10 years, since 1965, when Chairman Mao issued the "great call": "in medical and health work, put the stress on the rural areas." The results, although now taken for granted, have been amazing and unequalled anywhere else.

Child mortality has been reduced in a spectacular fashion, and something that seems to be a nationwide family planning programme has been set up. In a truly revolutionary process, the Western 'medical monopoly' has been overturned, yet its achievements have been incorporated into a truly national medical system that has drawn both from the experience of countless generations of traditional practitioners, and from modern science.

The term "barefoot doctor" is now known the world over. It seems that it was first used in the Chiangchen People's Commune on the outskirts of Shanghai in 1965, where a medical team started teaching young farmers to perform some medical tasks. These farmers continued to work in the fields, but also cared for their fellow farmers, who affectionately called them "barefoot doctors". A Chinese delegate at the 27th World Health Assembly has said that since then, about 1 million barefoot doctors, of different levels adapted

to the needs of their community, have been trained in China.

In developing countries, medical doctors tend to congregate in the cities, largely leaving rural areas unattended, with the exception of occasional visits or vaccination campaigns. In China, many mobile medical teams tour the countryside, transporting with them especially designed equipment that allows even major surgery to be performed 'in the field'. They attend to the most serious cases, teach, and give refresher courses to barefoot doctors.

An important aspect of this programme was pointed out at the World Health Assembly by Dr Wang Kueichen, a 37-year-old barefoot doctor—"There is no barrier between the doctor and the patient"—white coats, impersonal offices, gleaming instruments and the medical language, all of which often contribute to isolate the patient in the West, play no part in this patient-doctor relationship. Barefoot doctors, she pointed out, are called 'doctors', not paramedicals or auxiliaries; but the relationship is close enough, she said, for her usually to be called "little sister" or "little daughter".

The major concern is with preventive medicine, and this may well be the principal reason of the system's success. Basic preventive medicine, immunisation, health education, well-baby clinics, and surveillance of communicable diseases are enough to cut down radically the incidence of the most common illnesses in rural areas. Labour-intensive techniques have been used effectively against epidemics. For example, millions of schistosomiasis-bearing snails have been picked by hand and destroyed, and there have been similar campaigns against bedbugs, mosquitoes, rats, and flies. As one medical professor has put it: "Each of us, regardless of whether he was a janitor or university president, had to carry around a big fly swatter and a vengeful lust for dead flies. Everyone had his or her quota to fulfil by the end of the week."

This did not exclude curative medicine. The recognition and treatment of diseases such as respiratory infections and diarrhoea, which are among the most widespread causes of infant mortality, is in most cases a simple task, which requires neither a medical degree nor eight years of medical training. In such instances barefoot doctors practise what is referred to as "the New Chinese medicine", an integration of western and traditional procedures including acupuncture, herbal treatment, modern drugs and sometimes relatively complicated surgical procedures such as appendectomies. According to most reports, female barefoot doctors also routinely perform abortions, using the aspiration method. □

international news

THE Atomic Energy Commission (CEA) in France has reached the most important turning point in its history since its creation in 1945. On August 6, in a decision running counter to demands expressed by industrial syndicates that the total nuclear sector be placed under public responsibility, M Valéry Giscard d'Estaing approved the separation of the production side of the atomic energy industry in order to form a limited private company, a wholly owned subsidiary of the CEA.

In practice, the Directorate of Production constitutes a homogeneous entity with a wide range of activities, providing the military sector with strategic nuclear equipment and the public sector with all the services required for the fuel cycle—ores, enrichment, fabrication of fuel, treatment of irradiated fuel, storage of waste and so on. With the acceleration of the French nuclear programme, the resources of this department of the CEA have grown rapidly during the past few years, and in 1974 it even managed to defray 59.2% of its own costs. Because of budgetary and human imbalance in this sector as compared with the whole of the CEA, it follows quite naturally that it should become an industrial company.

At the same meeting, President Giscard d'Estaing agreed that the CEA, through the new company, should buy a stake in Framatome, now the sole construction company for nuclear reactor steam generators in France. Since 1973, two companies making nuclear generators have been competing for the French market, Framatome (a multinational corporation, 51% of which is owned by Creusot-Loire, 45% by the American Westinghouse group, and 4% by the Empain-Schneider group) and

Restructuring the French nuclear industry

from the staff of La Recherche

the General Electricity Company (CGE). The former is licensed by Westinghouse to use the pressurised water method. The government, going back on its two-year-old decision to go nuclear, decided at the beginning of the year to slow down the projected generation of nuclear energy. Since then, profiting partly from its former position in the French market and partly from a larger slice of the French industrial cake, Framatome has come through as the sole constructor of nuclear reactors. The government now has to negotiate the purchase of some of the interests of the American company. Although nothing is known about the talks in progress with Westinghouse, it seems that it might be persuaded to keep only an 11–12% stake in Framatome, which would give the CEA a minority block (with veto power) within the company.

Another industrial rearrangement adopted at the August 6 meeting is the setting up of a common structure between Framatome and the two companies making turbo-alternators in France, CGE and CEM (the latter a subsidiary of the German-Swiss group Brown-Boveri). As a result they can offer the world entire nuclear reactors, complete and ready to go.

The official arguments behind the new decisions are clear. The arrangements provide independence from the American license, gallicise the electro-

nuclear industry, create a powerful, competitive company to compete in the world construction market, and provide control over exports.

Industrially, the arguments are also very clear, but present distinctly divergent points of view, reflecting three main lines of thought. First, in gallicising American technology, and thereby raising France to the rank of a partner, the result will be to make available the multinational Empain-Schneider-Westinghouse group the scientific, technical and financial potential of a public body.

Second, the creation of a production subsidiary actually prolongs the gradual breaking up of the CEA, already initiated several years ago in the field of computing. The government has formed a limited company with state capital from a body which, having obtained resources for 20 years from the public sector, had arrived at a peak as regards work done on the fuel cycle. Though the principal client in France will still be the EDF, the French electricity authority, there is no benefit, either technical, industrial or even commercial, in forming this subsidiary company, unless it is derived from a progressive entry into the multinational market and from the involvement of other private groups, in particular of the Pechiney-Ugine Kuhlmann group, which is already a rival in the fuel cycle area.

Finally, the industrial groups view this gradual movement towards the formation of private companies with concern, lest the laws governing profit should impinge on production matters. In a phase of fast industrial development, where considerations of profitability and production predominate, the position of workers will remain at risk.

A DISCOVERY which, if confirmed, would rank as a major milestone in physics and which would upset some of the most sacrosanct physical equations, was reported in the United States last week. Physicists at the University of California at Berkeley and at Houston University believe they have detected a magnetic monopole in the upper atmosphere.

The evidence comes from a track made in a package of plastic sheets and a photographic plate flown from a balloon at 130,000 feet in 1973. The belief is the track was made by a magnetic monopole with a magnetic

charge of 137 and a mass of more than 200 times that of a proton, which was travelling toward the Earth at

Monopole evidence

about half the speed of light.

One of the team, P. Buford Price said last week that "from any piece of our evidence you might conclude a different particle passed through our detector. But all the findings put together force the conclusion that it was

a monopole. There is no other known explanation".

First predicted by Dirac in 1931, magnetic monopoles have been hunted intensively and their possible existence has been hotly debated ever since. Physicists have anticipated confirmation of their existence with mixed feelings, since although they would add symmetry to the world of particle physics—they would be the magnetic equivalent of electrons and protons—their existence is incompatible with the Maxwell's equations, which have stood since 1865 as the basic equations of electromagnetism.

THE success of the Ariel-5 satellite has produced an unfortunate outburst of parochialism in some sections of the British press, with comments such as "Britain leads the world in X-ray astronomy" and "most discoveries are British" recurring among reports of the latest discovery. This may be true at the present moment, since the US Uhuru satellite is no longer effective, plans for a European X-ray astronomy satellite have yet to be finalised and clearly the UK national press finds the Netherlands too small to notice. But the reaction is a particularly unfortunate one in view of the great importance of international cooperation in X-ray astronomy, and the great success of that cooperation so far.

Data from the pioneering Uhuru satellite, for example, were made widely available outside the circle of US experimenters directly concerned with its design and operation; the widely publicised Copernicus satellite combines both US ultraviolet telescopes and UK X-ray detectors; and indeed Ariel-5 itself carries a US experiment, and was, come to that, launched by a US rocket. The series of letters beginning on page 628 of this issue gives some idea of the extent of international cooperation in this sophisticated area of research; an even better indication is provided by the four letters on pages 107-112 of this volume, in which groups from three countries using observations from four

satellites and from the ground combine to describe a change in the X-ray and radio emission from the source Cygnus X-1.

Of course there is an air of friendly rivalry between the different groups, especially in the urge to publish observations of an exciting new discovery first; but everyone involved in X-ray

Astronomy in Britain

astronomy is well aware of the need to make the best use of the limited funds available. This will not be achieved if we move towards a situation where the rivalry becomes real and deep, with data being hugged close to the chests of the discoverers while they indulge in mutual claims that "my satellite is better than yours". It is to be hoped that the discoverers of exciting new phenomena in space will in future, when they announce how remarkably clever they have been to find anything at all, encourage the listening reporters to present a balanced view of the remarkably successful international collaboration behind the whole story. To many people, it is more significant to learn that scientists from different parochial groups can work together in effective harmony than to learn that something has been discovered and that no one quite knows what it is, but at

least it was a British discovery.

● Minor Planet Circular 3827 of the International Astronomical Union announces the names accorded to a number of Minor Planets discovered by Dr L. Kohoutek with the 80-cm Schmidt Camera of the Hamburg Observatory, Bergedorf. A singularly appropriate one is MP 1896, named Beer, in honour of Dr Arthur Beer, formerly of the Cambridge Observatories, who recently celebrated his 75th birthday. The citation refers particularly to his 20 year editorship of the series *Vistas in Astronomy*, first produced in 1955 in honour of Professor F. J. M. Stratton, but continued as a serial publication that is highly regarded by astronomers for the wide ranging nature of its articles, essays and reviews. Dr Beer joins the small number of astronomers in England with a planet named after them in their lifetime—others were Stratton, and Dr G. Merton of Oxford.

The same Circular names planets 1897-1899 after J. R. Hind, the English astronomer who discovered 10 minor planets in the middle of last century, and after P. H. Cowell and A. C. Crommelin, distinguished in this century for their contributions to computational methods in minor planet and comet theory. Together they investigated the orbit of Halley's comet and identified its apparitions back to 239 BC.

UNDER a new agreement between the United States and Israel a water desalination project likely to cost \$55 million has just been started. A test module is to be built at the Mediterranean port of Ashdod, about 40 km south of Tel-Aviv, to be followed by a prototype plant with a desalting capacity of 10 million gallons a day, linked with the local Eshkol power plant. America's contribution will amount to \$20 million.

American interest in Israeli desalination has a long history. Section 219 of the Foreign Aid Bill approved by Congress in 1969 provided for the joint participation of the U.S. government and the State of Israel in the development of a large desalination plant, including the construction of a prototype for such a plant and its test operation. For this the sum of \$20 million dollars was allotted by Congress.

In February 1971 the Israeli National Council for Research and Development (NCRD) presented to the US, on behalf of the Israeli government a detailed draft of a proposed Multi-Effect Distillation process (MED), developed by Israel Desalination Engineering Ltd (IDE), and backed by NCRD since 1970 as

Israel and US in water deal

from Kapai Pines, Jerusalem

probably the best desalination system in the world.

In November 1972 a memorandum of understanding was signed between both governments to construct an MED prototype plant at Ashdod, and on May 13 this year the US Secretary of the Treasury and the Israeli Minister of Finance signed a joint statement in Washington while meeting at a US-Israel Joint Committee for Investment and Trade. They said that the proposed joint water desalination project had undergone a lengthy period of evaluation, and they agreed that it was now feasible to proceed with the arrangements for the design, construction and initial operation of a large-scale prototype plant and to negotiate a technical agreement subject to the necessary consultations with Congress. Subsequently, a US technical mission came to Israel, and on May 21 a joint agreement was negotiated between the two countries to carry out the project.

A small MED pilot-plant inaug-

urated near Eilat in June last year, produces about 1 million gallons of potable water per day. This water is integrated into the regular water supply system of Eilat, and the technology of this plant forms the basis for the proposed large-scale plant in which the Americans are interested. The new distillation process is said to cut the cost of desalted water from 60 American cents per 1,000 gallons in other distilling processes to less than 24 cents. One of the distinguishing advantages of the IDE process is that it can efficiently employ low-pressure, low-temperature steam, while other distillation processes require higher temperatures for efficient operation. Furthermore, the overall process is characterised by maximum energy recovery and minimal waste.

Another distinguishing feature resulting in economic advantage involves the use of aluminium tubing rather than copper in the fabrication of heat exchangers. The use of the aluminium is made possible by the relatively low temperature of the process, and also results in a substantial savings, since heat exchangers comprise a major cost-element in plant construction. □

news and views

Tumour virus proteins at the cell surface

from Reinhard Kurth

THE enveloped RNA-containing viruses are oncogenic for a wide variety of species, of which the avian, murine and feline systems have received considerable attention. Following the discovery that these viruses replicate by reverse transcription by way of a DNA provirus (Baltimore, *Nature*, **226**, 1209, 1970; Temin and Mizutani, *Nature*, **226**, 1211, 1970), it was shown by nucleic acid hybridisation that all cells of the species tested contained chromosomally integrated provirus information. Depending on the genetic background of the host, the interaction between endogenous virus and host cell can lead to different states of virus expression, ranging from complete repression, partial expression of some virus polypeptides (VPP) to complete particle production. Silent endogenous viruses can often be activated by (mis-) treating cells with chemical or physical agents (Lowry *et al.*, *Science*, **174**, 155; 1971; Aaronson *et al.*, *Science*, **174**, 157; 1971). The question why these and possibly all animals (and humans?) harbour endogenous virus genomes is a mystery, which may now be gradually resolved.

If endogenous virus genomes were consistently silent with no potentially harmful effects, it would be understandable that no selective evolutionary pressure has operated against them. As mentioned above, this does not seem to be the case: provirus information can be translated into VPP. Gross obtained evidence a long time ago that a vertically transmitted and *in vivo* activated murine leukaemia virus was indeed oncogenic (*Proc. Soc. exp. Biol. Med.*, **76**, 27; 1951). Recent work by Aaronson's group seems to indicate also that *in vitro* activated endogenous viruses can be oncogenic (Stephenson *et al.*, *J. Virol.*, **13**, 237; 1974; Greenberger *et al.*, *Cancer Res.*, **35**, 245; 1975). Why then has natural selection failed to eliminate this information from the cell?

The answer could lie in some new data which indicate that endogenous viral information may also become activated *in vivo* under specific and so far little characterised circumstances to play a part in differentiation and/or in the prevention of oncogenesis by exogenous oncornaviruses. In such

specific instances, the expression of endogenous viral information may confer a selective advantage for the host.

Progress in the understanding of virus-host cell interactions has advanced with the isolation of the major structural VPP (see table). The individual VPP are biochemically and immunologically distinct, the only cross-antigenicity existing between the glycosylated envelope proteins of a given virus strain. On the other hand (glyco-) proteins of the same size are biochemically very similar and cross-antigenic for virus strains of the same or related species. They exhibit both group-specific and interspecies-specific antigenic determinants in addition to their distinct virus type-specific antigens (reviewed recently by Bauer, *Adv. Cancer Res.*, **20**, 275; 1974).

Plasma membrane of tumour cells

For the sake of this discussion, it is necessary to distinguish between VPP inserted into the surface membranes of uninfected and of oncornavirus-infected cells. For more than a decade it has been known that virus envelope glycoproteins (gp85 or gp69/71) are often inserted into the membranes of oncornavirus-infected cells, which is not too surprising as these viruses bud from the host cell membrane. What is unexpected, however, is the more recent finding that p30, the major structural internal protein of oncornavirus particles, can also become inserted into the plasma membranes of exogenously infected cells (Ferrer, *Int. J. Cancer*, **12**, 378; 1973; Yoshiki *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1878; 1973; and *J. exp. Med.*, **139**, 925; 1974). These first observations have now been confirmed and extended by several other laboratories to show that in addition to the large envelope glycoproteins, p30, as well as the small VPP p15, p12

and p10 can be expressed either alone or in different combinations on the surfaces of a wide variety of tumour cells (Friedman *et al.*, *J. Virol.*, **14**, 1126; 1974; Ikeda *et al.*, *J. Virol.*, **14**, 1274; 1974; Billelo *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3234; 1974; Moroni *et al.*, *Intervirology*, **3**, 292; 1974; Knight *et al.*, *Int. J. Cancer*, **15**, 417; 1975; Del Villano *et al.*, *J. exp. Med.*, **141**, 188; 1975; Hunsmann *et al.*, *Virology*, in the press; Schwarz *et al.*, *Virology*, in the press).

Furthermore, some chemically induced mouse tumours can express gp69/71 and low levels of p30, p15, p12, and p10 at their surface (Grant *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 5037; 1974; Ikeda *et al.*, *J. Virol.*, **14**, 1274; 1974; Yoshiki *et al.*, *J. exp. Med.*, **139**, 925; 1974). In these instances the VPP must be coded by activated provirus. These observations extend earlier data on endogenous virus activation by physical and chemical agents (reviewed by Todaro, *Persp. Virol.*, **8**, 81; 1972).

On the other hand, transformation events do not always lead to the insertion of viral products into the plasma membrane (Rapp *et al.*, *Virology*, **65**, 392; 1975) and we know little about what (genetic) factors determine the transformed phenotype. Qualitatively similar results have also been obtained in the feline (Yoshiki *et al.*, *J. exp. Med.*, **139**, 925; 1974; Ikeda *et al.*, *J. Virol.*, **14**, 1274; 1974; Essex, personal communication) and the avian (Kurth and Bolognesi, unpublished) oncornavirus models.

Plasma membrane of normal cells

The detection of non-glycosylated VPP on the transformed cell surface cannot easily be explained. Why are they there? What is their function, if any?

Major structural polypeptides of oncornaviruses

Species	Virus polypeptides (VPP)							
	gp85†	gp37	p27§	p19	p15	p12	p10	
Avian*								
Murine*	gp69/71	gp45	p30	p19	p15	p12	p10	
Feline†	gp100	gp70	p30	p21	p15	p11	p10	

*† Data from: *August, Bolognesi, Fleissner, Gilden, and Nowinski, *Virology*, **60**, 595; 1974. †Essex, *Adv. Cancer Res.*, **21**, in the press.

‡gp85 = glycoprotein of molecular weight 85,000.

§p27 = non-glycosylated protein of molecular weight 27,000.

The attempt to find a general explanation is further complicated by the recent discovery that certain normal, uninfected cells also synthesise VPP which are expressed in their plasma membrane.

Experiments with tumour cells and monospecific sera directed against individual VPP have often given background reactions with normal mouse, rat, cat or chicken fibroblasts (Grant, Ikeda, Yoshiki, cited above; Hunsmann *et al.*, *Virology*, in the press; Schwarz *et al.*, *Virology*, in the press; Kurth and Bolognesi, unpublished; Hogg, personal communication). Some groups decided to neglect the normally weak antiserum reactivities by preabsorbing their sera on normal fibroblasts. This approach may be justified, as the purified VPP used for immunisation may have been slightly contaminated with normal cellular material. It is, however, just as conceivable that normal fibroblasts express some VPP, perhaps in a form structurally modified by the host cell. Data obtained in the avian oncornavirus system seem to support the latter interpretation; gp85 and p27 seem to be present in comparatively high quantities in the membranes of most if not all uninfected chicken fibroblasts derived from embryos of various flocks (Smart, Bosch, Kurth and Bolognesi, unpublished).

A role in differentiation?

It is not surprising that normal lymphoid tissue from high leukaemia mouse strains express gp69/71 and p30 in their membranes (Yoshiki *et al.*, *Proc. natn. Acad. Sci. U.S.A.* **70**, 1878; 1973; Kennel *et al.*, *Virology*, **55**, 464; 1975; Del Villano *et al.*, *J. exp. Med.* **141**, 172; 1975), as these cells might be considered premalignant. Less expected, however, are the recent results obtained by the virologists of the Scripps Clinic: they now find gp69/71 not only on mouse lymphoid cells, but also on certain foetal and adult tissues, notably epithelial cells (Lerner, Wilson, Del Villano, McConahey and Dixon, unpublished). The data indicate that the membrane expression of endogenous VPP might follow a precise pattern with respect to tissue distribution and kinetics of appearance/disappearance. It has been suggested repeatedly for various animal model systems that morphogenesis and subsequently the maintenance of a differentiated state could be mediated (among other factors) by cell surface receptors (for reviews see *Humoral Control of Growth and Differentiation*, Academic Press, New York, 1973). If endogenous VPP are indeed among those receptors possessing a transmitter function in the exchange of information between cell and environment,

they would have a role in the induction and maintenance of established morphogenetic patterns. Such a role could explain the positive selective advantage in having potentially oncogenic endogenous virus genomes integrated into the cellular genome.

At this point, one is left with a complex situation. On one hand, VPP coded by endogenous or exogenous oncornaviruses can become expressed on the transformed cell surface and could therefore be considered as tumour-specific antigens. On the other hand, certain normal tissues also seem to express endogenous membrane VPP. The picture is further complicated by the concomitant presence of natural antibodies directed against endogenous VPP (Hanna *et al.*, *Cancer Res.* **32**, 2226; 1972; Ihle *et al.*, *J. exp. Med.* **138**, 194; 1973 and *J. exp. Med.* **139**, 1568; 1974; Lee *et al.*, *J. Virol.* **14**, 773; 1974; Aaronson and Stephenson. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1957; 1974). This situation is reminiscent of tumour-bearing animals in which tumours and their specific antigens coexist with anti-tumour antibodies and lymphocytes.

Anti-tumour response

Could VPP on tumour cells be used as tools in vaccination or immunotherapy? This seems unlikely as immunisation with isolated VPP could greatly enhance the risk of autoimmune diseases or glomerulonephritis. In fact, Hanna and coworkers (*Cancer Res.* **32**, 2226; 1972; *J. natn. Cancer Inst.* **52**, 117; 1974) have described a correlation between the development of glomerulonephritis and the persistence of high titres of natural antibodies.

In contrast, there are experimental situations in which the anti-tumour response is clearly mediated by VPP located in the transformed cell surface (Knight, Mitchison and Shellam, *Int. J. Cancer*, **15**, 417; 1975; Gorczynski and Knight, *Br. J. Cancer*, **31**, 387; 1975) or in which immunisation with exogenous gp69/71 leads to significant anti-tumour protection (Hunsmann, Moenning, and Schäfer, *Virology*, in the press). A comparison of the antigenic determinants accessible to antibodies on the cell surface (not necessarily of the VPP molecules in the cell membrane) with antigenic sites in the viral particle may resolve these discrepancies. There is preliminary evidence that endogenous VPP in the cell membrane may have undergone somatic cell modification (Del Villano *et al.*, *J. exp. Med.* **141**, 172; 1975; Tung *et al.*, *J. exp. Med.* **141**, 198; 1975). Thus it may turn out that the anti-tumour response is (predominantly) directed against antigenic sites restricted to exogenous VPP of the

transforming virus which are not concomitantly expressed by endogenous membrane VPP in normal cells. For both theoretical and practical reasons, this problem deserves further clarification.

Control of VPP expression

A final question concerns the control of endogenous virus expression, especially in differentiating tissue. There are at least two model systems where this question can be studied further. First, recent work from Boyse's laboratory at the Memorial Sloan-Kettering Cancer Center proved convincingly that the G_{IX} antigen on normal thymocytes of certain mouse strains (Stockert, Old and Boyse, *J. exp. Med.* **133**, 1334; 1971) is biochemically and immunologically related and possibly even identical to Gross leukaemia virus gp69/71 (Obata *et al.*, *J. exp. Med.* **141**, 188, 1975; Tung *et al.*, *J. exp. Med.* **141**, 198, 1975). The second model exists in the avian system, where the helper activity of normal chicken fibroblasts to complement the defective Bryan high titre strain appears to be correlated with the synthesis of a chicken helper factor thought to be gp85 (Payne and Chubb, *J. gen. Virol.* **3**, 379; 1968; Weiss and Payne, *Virology*, **45**, 508; 1971).

In general, the membrane insertion of individual VPP seems to be a non-coordinate event; different combinations of VPP have been detected on normal and transformed cells. Their expression appears to be governed by autosomal dominant genes (Strand, Lily and August, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3682; 1974), just as in the G_{IX} and chicken helper factor models. (For recent reviews see Lilly and Pincus, *Adv. Cancer Res.* **17**, 231; 1973; Weiss, *Persp. Virology*, **9**, 165; 1975.)

It has been a drawback for progress in cell surface immunology, including tumour immunology, that many of the groups involved were predominantly occupied with the mere description of the distribution of cell surface moieties. Very few attempts have been made to investigate their function, for example with respect to enzymatic activities or transport processes. Since it is now possible to purify endogenous or exogenous VPP in milligram quantities, one may soon be able to test them for possible functions which may explain their cell surface location. Additional data on the distribution of endogenous VPP in different organs and the kinetics of their appearance and disappearance in differentiating tissues may establish further associations between the expression of a virus-related phenotype and the process of cellular differentiation. □

More RNAs for oocytes

from Pamela Hamlyn

ALTHOUGH *Xenopus laevis* oocytes are interesting in themselves as a means of studying gene action, their more familiar role is that of an assay system for the translation of injected messenger RNAs. They have many advantages for this purpose, not least their ready availability—several thousand mature oocytes from one adult female. (Compare this with scanning the shelves of health food shops for just the right wheat germ.) Injected messenger RNA is translated with a high efficiency, globin mRNA being translated at nearly the same rate as in intact reticulocytes. Because of this very high efficiency only minute amounts of mRNA are required to produce detectable amounts of protein product and consequently mRNA activity can be detected even when crude RNA fractions containing very little mRNA are injected into oocytes.

van der Donk made use of this property in some experiments that are reported in this issue of *Nature*, **256**, 674; 1975). He is interested in the mechanism by which the style of *Petunia hybrida* is able to recognise and to differentiate between its own pollen and that of another plant. (Pollen tubes only grow after cross-pollination.) Total RNA extracted from self-pollinated styles stimulated

protein synthesis (by about three times) when injected into *X. laevis* oocytes. A fractionation of the RNA according to molecular weight showed that it was the RNA between 5S and 18S that gave the biggest stimulation. But stimulation of protein synthesis is completely inadequate as proof that the injected RNA is being translated. Stimulation of endogenous protein synthesis cannot be ruled out as an explanation unless the protein for which the injected messenger codes is shown to be synthesised in the oocytes. The endogenous protein synthesis in the oocyte is higher than in some cell-free systems. In spite of this disadvantage van der Donk was able to demonstrate the appearance of several new protein bands on gel electrophoresis of proteins synthesised in oocytes injected with plant RNA. Nonetheless it could be argued that these bands were oocyte protein whose *de novo* synthesis was stimulated by the plant RNA. To identify them as plant proteins van der Donk has attempted to demonstrate their function in the inhibition of pollen tube growth. Protein can be isolated from styles after self-pollination which, when transferred to a style that has been cross-pollinated, will inhibit the growth of pollen tubes in that style. Therefore a solution of proteins synthesised in oocytes in the presence and absence of RNA isolated from self-pollinated styles was applied to styles that had been cross-pollinated. Only the proteins synthesised in the presence of the plant RNA were able to inhibit the growth of the pollen tube. Van der Donk takes this as a clear demonstration that at least part of the proteins translated from plant RNA are those involved in the incompatibility reaction (the inhibition of pollen tube growth), and that by partially isolating the RNA coding for these proteins a further step has been made in the elucidation of the intriguing mechanism by which a plant distinguishes between self and non-self.

Gurdon and his colleagues have demonstrated the very long life—up to 10 days—of globin mRNA after injection into oocytes (Gurdon, Lingrel and Marbaix, *J. molec. Biol.*, **80**, 539; 1973). Allende and coworkers reasoned that this must be because oocytes contained a very low level of ribonuclease activity or else the RNA was protected in some way from degradation (Allende, Allende and Firtel, *Cell*, **2**, 189; 1974). To distinguish between these two possibilities they injected a variety of radioactive natural and synthetic RNAs into oocytes and followed their degradation with time. They were able to show that some RNAs were degraded and others were not, and concluded that the oocytes contained enough ribonuclease activity to degrade

injected RNAs so that those remaining must be protected in some way. They next focused their attention on yeast tRNA—one of the species that survived undegraded in oocytes even after 20 h. In this issue of *Nature* they report their demonstration that oocytes are capable of aminoacylating yeast tRNAs injected into cells even at final concentrations which were much greater than that of their endogenous tRNA content (page 675). They showed that oocytes can be injected with tRNA for phenylalanine to a final concentration in the cell 500 times the normal concentration—acylation taking place so that there is 150 times as much Phe-tRNA as normal. The authors plan to study the effects of this imbalance on the mechanism of protein synthesis.

Nuclear spectroscopy

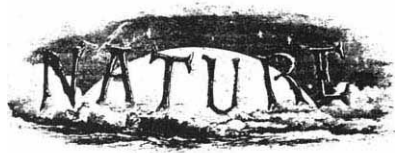
from P. E. Hodgson

IN a nuclear transfer reaction angular momentum is conserved vectorially, so that the spin of the final state of the residual nucleus $J_R = J_T + j$, where J_T is the spin of the ground state of the initial (or target) nucleus and j the angular momentum of the transferred nucleon. This j is itself composed of its orbital and spin angular momenta, $j = l + \frac{1}{2}$. The angular distribution of the outgoing particle, for example that of a proton in a (d,p) reaction, is usually characteristic of the orbital angular momentum transfer l , and thus l can be determined. Since J_T is known, this suffices to set limits on J_R and to determine it in some special cases.

For example, if $J_T = 0$, $J_R = j = l + \frac{1}{2}$, so that $J_R = l \pm \frac{1}{2}$. So if $l = 2$, the final state is $d_{3/2}$ or $d_{5/2}$. This ambiguity may be resolved either by rather difficult measurements of the polarisation of the outgoing particle, or by careful examination of some special features of the differential cross sections (*Nature*, **250**, 464; 1974; **253**, 501; 1975).

The situation is more complicated if J_T is greater than zero, for then several values of j may be possible in reactions to states of the same J_R . Thus if $J_T = \frac{1}{2}$, $J_R = 1$, and $l = 1$, then j can be $\frac{1}{2}$ or $\frac{3}{2}$. It is an interesting problem to determine j , for if this can be done we can learn more about the reaction and also use such reactions to determine J_R in cases where it is not known. Furthermore, j has to be known in order to apply the j -dependent sum rules to determine nuclear spectroscopic factors (*Nature*, **249**, 695; 1974).

Some years ago Kocher and Haeberli (*Phys. Rev. Lett.*, **23**, 315; 1969) showed that the vector analysing power in one-nucleon transfer reactions is



A hundred years ago

AMONGST the objects which have been recently added to the galleries of the Paris Industrial Exhibition of Geography, and are attracting public notice, we may mention a collection of French birds exhibited by M. Bouvier, the collection of apes from the Gaboon, by the Marquis de Compiègne, and a number of antediluvian fossils from the Mentone Caves. The skeletons of two children which had been buried together are in a splendid state of preservation, exhibiting admirably the characteristics of prehistoric cave-life. These two young people were buried in the home of their parents, very probably because it was the only means of defending their bones against the teeth of ferocious hyenas and other large carnivorous animals which were disputing with man the empire of the future Gaul.

from *Nature*, **12**, 358; August 26, 1875.

sensitive to the value of j and thus provides a good way of determining it. They obtained the vector analysing power from the left-right asymmetry of the protons emitted from a reaction initiated by a polarised beam of particles. The $j=\frac{1}{2}^-$ reactions $^{52}\text{Cr}(\text{d,p})^{53}\text{Cr}$ ($Q=5.17$ MeV) and $^{54}\text{Fe}(\text{d,p})^{55}\text{Fe}$ ($Q=6.60$ MeV) at 10 MeV had vector analysing powers quite different from the $j=3/2^-$ reactions $^{52}\text{Cr}(\text{d,p})^{53}\text{Cr}$ ($Q=5.73$ MeV) and $^{54}\text{Fe}(\text{d,p})^{55}\text{Fe}$ ($Q=7.01$ MeV) at 10 MeV. In the mixed- j reaction $^{53}\text{Cr}(\text{d,p})^{54}\text{Cr}$ ($Q=6.71$ MeV) at 10 MeV, both these values of j were possible, and the vector analysing power closely followed a curve obtained by adding together in definite proportions the vector analysing powers found in the other two reactions. This proportion gave the relative contributions of the $j=\frac{1}{2}$ and $j=3/2$ components to the reaction. The technique of finding the mixing parameter in mixed- j transitions has the special advantage of being independent of detailed theoretical calculations, but is somewhat laborious to apply in practice.

A new method of finding the j value has recently been proposed by Dohan and Summers-Gill (*Nuclear Phys.*, **A241**, 61; 1975), and applied to the $^{69}\text{Gd}(\text{d,p})^{70}\text{Gd}$ and $^{71}\text{Gd}(\text{d,t})^{70}\text{Gd}$ reactions to the same state of ^{70}Gd . It depends on the fact that, on the simple shell model, the $2p_{1/2}$ neutron state is empty in ^{69}Gd , half-full (containing one neutron) in ^{70}Gd and full in ^{71}Gd . Thus the (d,p) and (d,t) reactions on ^{69}Gd and ^{71}Gd respectively go equally well, and thus have very similar spectroscopic factors. The $2p_{3/2}$ state, on the other hand, is full for all these nuclei, so the (d,p) reaction on ^{69}Gd is forbidden, as there is no room for another neutron, while the (d,t) reaction on ^{71}Gd takes place easily as there are plenty of $2p_{3/2}$ neutrons to be removed. This picture is expected to be somewhat blurred as the simple shell model is not followed exactly, but nevertheless we expect to find very similar amplitudes for $2p_{1/2}$ transfer and very different amplitudes for $2p_{3/2}$ transfer in the two reactions.

In practice these expectations are remarkably well fulfilled. The transitions to the lower states up to about 1 MeV have almost the same amplitudes for the two types of reaction while those above 1 MeV have very small amplitudes for the (d,p) reaction and large amplitudes for the (d,t) reaction. Thus we can with some confidence assign the first group to $2p_{1/2}$ transfer and the second group to $2p_{3/2}$ transfer. This is a very simple and direct way of determining the j value of the transferred particle, and is applicable to reactions where the sub-shell corresponding to one j value is filling more rapidly than the other. It

will be interesting to see how far it can be extended.

In heavy ion reactions the situation is more complicated since the transferred particle can have an angular momentum greater than zero in the projectile. Thus the neutron in the deuteron has $l=0$, whereas the proton in ^7Li that is transferred by the ($^7\text{Li},^6\text{He}$) reaction has $l=1$, $j=3/2$ as it comes from an $p_{3/2}$ orbit in ^7Li (*Nature*, **253**, 501; 1975).

Sticky actin

from Dennis Bray

MUSCLE biochemistry is not usually the place to look for the bizarre; but what else could one call an association between muscle actin and an enzyme that digests DNA? The story is a curious one. As long ago as 1943, it was found that pigeon thymus contains a protein that inhibits deoxyribonuclease I—the abundant endonuclease of pancreas. This activity was found in other tissues but little else was learnt about its biochemistry until the late 60s when Lindberg—at the Karolinska Institute, Stockholm—began a careful study. He found that the inhibitor had a molecular weight close to 42,000; that it could be purified and even crystallised by conventional procedures; and that it formed a tight 1:1 complex with the nuclease. Among its notable characteristics, at least in retrospect, was its abundance in tissue (5–10% of the protein in a soluble extract), and its tendency to form aggregates of high molecular weight.

Then last year, at Cold Spring Harbor, New York, Lazarides and others interested in cell motility heard at first-hand of the DNase inhibitor. They also had a protein with molecular weight close to 42,000 which was strangely abundant in cells: only they called it actin. The amino acid compositions of the two proteins were encouragingly similar and the unlikely possibility was put to the test. Sure enough, the fingerprints of the two are the same, they cross react immunologically, and analytical-grade muscle actin sticks so tightly to DNase I that denaturing agents are needed to part them (Lazarides and Lindberg, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4742; 1974).

The interaction is unquestionably real but why does it occur? It is unlikely to be accidental because the binding is so tight. It may have some purpose, but it links two cellular processes that are normally considered to be independent. The puzzle is compounded by indications that actin forms other unexpected associations. Before the work mentioned above, Laki and Muszbek (*Biochim. biophys. Acta.*,

371, 519; 1974), had shown that actin in its filamentous form binds strongly to fibrin—the major structural protein of blood clots. And a recent paper examines the long known association between glycolytic enzymes, such as aldolase and triose phosphate dehydrogenase, and filamentous actin (Clark and Masters, *Biochim. biophys. Acta.*, **381**, 37; 1975). Still only in abstract form are claims that an interaction exists between actin and spectrin, the major protein of red blood cell ghosts (Tilney, *J. Cell Biol.*, **63**, 349a; 1974); and another report that actin might be identical to the γ -subunit of dogfish muscle phosphorylase (Fischer *et al.*, *Hoppe-Seylers Z. Sür Physiol. Chem.*, **356**, 381; 1975).

If we consider the proteins that associate with thin filaments in muscle—tropoin, tropomyosin, myosin and actinin—then we have ten or so that all bind to actin. The list might well be longer, for any club that includes both deoxyribonuclease and myosin must surely have a wide membership. It begins to look as though we are in the presence of a Phenomenon; but what it might mean is hard to say. Either actin is generally sticky and able to adhere to proteins it does not normally encounter, or many proteins have evolved binding sites that hitch them to this abundant and phylogenetically stable component of the cell.

Evolution of *E. coli* chromosome

from Millicent Masters

It has been possible to observe the intact chromosome of about half-a-dozen species of bacteria. In each species the DNA was found to be a closed circle. The circular chromosome of the best studied species of bacterium, *Escherichia coli*, contains enough DNA to code for about 3,000 average-sized polypeptide chains. Specific functions have been assigned to about 500 of these genes and the well developed genetics of *E. coli* has permitted their positions on the chromosome to be determined. Genes specifying proteins with related functions such as isoenzymes catalysing the same reaction or groups of enzymes involved in sequential metabolic conversions may be either close together or distant on the circular chromosome. Some related genes are close together because they are transcribed together (that is, they form an operon). Other related genes however are close together without sharing such a common control mechanism. Still other related genes, such as the five genes concerned with pyrimidine synthesis or seven of the 11 genes responsible for arginine biosyn-

thesis, occupy widely disparate locations on the chromosome.

David Zipkas and Monica Riley in a recently published article (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1354; 1975) have examined the locations of 125 genes, each of which was judged to be biochemically related to at least one other gene among the 125. With the help of a computer they analysed the distances between all pairs of genes and between all those pairs judged to specify biochemically related functions. They found that pairs of related genes were not placed randomly relative to one another but, rather, that they were almost twice as likely to be located 90° or 180° apart than might be expected by chance. To explain this remarkable observation the authors propose that the chromosome of *E. coli* is the product of two successive duplications of a primitive genome which was one quarter the size of the present-day chromosome. This event, postulated of course to have occurred a very long time ago, would have resulted in a tetrameric structure with each gene present four times at 0°, 90°, 180° and 270° positions.

In support of this idea they cite Wallace and Morowitz (*Chromosoma*, **40**, 121; 1973). These authors tabulated the genome sizes of about 70 species of bacterium and found that the sizes, which vary from about 0.5 to 3.0×10^9 daltons, do not vary continuously. *Mycoplasma* species have chromosome sizes of either 0.5×10^9 or 1.0×10^9 daltons. The other species recorded have genome sizes clustered around peaks at 1.4×10^9 and 2.8×10^9 daltons. These provocative data are consistent with the idea that smaller genomes have given rise to larger ones by a process of genome duplication.

Zipkas and Riley further propose that the dimeric or tetrameric products of genome duplication evolved to their present forms by mutation of, and divergence in function among, the duplicate gene copies. For example, duplicate copies of an ancestral gene concerned with tryptophan metabolism could have evolved differently to yield in one case the gene for the tryptophan synthetase B protein and, 180° distant from it, the gene for tryptophanase. Both of these proteins can catalyse the conversion of indole and serine into tryptophan. Other cases however are more difficult to explain. The genes *arg F* and *arg I* which are neither 90° nor 180° apart both specify an ornithine transcarbamylase. Zipkas and Riley suggest that, although they specify proteins that carry out the same reaction, these two genes may have had independent origins (and as a consequence might be expected to have different sequences). Recent work, however, by Kikuchi and Gorini (reported at the

Fifth International Conference on the Biology of Temperate Bacteriophage, 1975) showing that the *arg I* and *arg F* genes have similar base sequences is inconsistent with this idea, and suggests that one of the genes arose by duplication of the other. Thus Zipkas's and Riley's suggestion that functionally related genes not 90° or 180° apart evolved independently is proved invalid in the one case where information on sequence homology is available.

It will obviously be difficult to either prove or invalidate the theory of Zipkas and Riley concerning the origin of the *E. coli* chromosome. Further information regarding sequence homology to support the necessarily somewhat arbitrary assignment of functional relationships will certainly be necessary. None the less, whatever the outcome of such studies, their provocative observation will remain to be explained.

Mitochondria and chloroplasts

from John Ellis

A pre-FEBS symposium on "The Structure, Synthesis and Functions of Nucleic Acids in Organelles" was held in Paris on July 19.

WITHIN the next year the complete genetic map of yeast mitochondrial DNA should be known. This bold prediction was made by P. Borst (Amsterdam University) whose optimism was generated by two recent technical advances. The first is the availability of restriction endonucleases, bacterial enzymes which cleave double-stranded DNA at specific base sequences into defined fragments which can be separated by gel electrophoresis. Hybridisation of the fragments with mitochondrial ribosomal RNA has revealed that the two ribosomal cistrons in yeast are not linked as they are in bacteria, but are located on opposite sides of the circular mitochondrial genome. This is not a constant feature of the mitochondrial genome however, since I. B. Dawid (Carnegie Institution, Baltimore) showed that the two cistrons are separated by a spacer region of only 120 base pairs in the mitochondrial genome of *Xenopus*, HeLa, and *Drosophila*.

The second advance is the production of a new class of mutations in the mitochondrial genome. P. Slonimski (Gif-sur-Yvette) pointed out that, with the exception of mutations that confer resistance to antibiotics, most mutations in the yeast mitochondrial genome were of the so-called *rho* minus type where

large deletions of the mitochondrial DNA have occurred. In such mutants the protein-synthetic system of the mitochondria is not functional, and so no genetic mapping is possible. What is needed is a class of point mutations where proteins continue to be made but which lack specific enzymic activities. Recombination between such structural gene mutations should restore wild-type mitochondria and hence permit genetic mapping. Slonimski reported the isolation of 120 such *mit* minus mutants; some confer normal cytochrome spectra but have no cytochrome activity, whereas others give abnormal spectra. Mapping to a resolution of 30–50 base pairs is in progress. It would be a mistake to think that there is a universal mitochondrial map however; Dawid pointed out that the mitochondrial DNA of sheep and goats differs by 80% in base sequence, thus lending substance to an old parable.

Restriction enzymes have also been used to study the DNA of the kinetoplast, an organelle related to the mitochondrion, and characteristic of trypanosomes and related protozoans. Each kinetoplast contains many thousand DNA minicircles of contour length 0.45 μ m, as well as longer linear molecules. G. Riou (Gustave-Roussy Institute, Villejuif) reported kinetic complexity measurements on restriction fragments of this DNA; the minicircles show about 2% heterogeneity in base sequence, while the linear molecules contain a sequence of kinetic complexity similar to that found in mitochondrial DNA. No genes have yet been located in the kinetoplast genome, nor is the significance of multiple minicircles understood.

Higher plant and yeast mitochondrial DNA has a contour length and a kinetic complexity of 25–30 μ m, while animal mitochondrial DNA is only 5 μ m in length. Why should more genes be needed in plant mitochondria than in animal mitochondria? Borst suggested that 5 μ m is sufficient for the structural genes required for all mitochondria, and that any extra length contained regulatory sequences. Slonimski amplified this notion by pointing out that lengths longer than 5 μ m were found either in facultative aerobes such as yeast, which might need more control sequences than obligate aerobes such as animals, or in green plant cells where the mitochondrion interacts metabolically with the chloroplast.

Chloroplasts from several higher plants and algae contain DNA circles of contour length about 45 μ m; this has been regarded as a universal size for the chloroplast genome but R. Herrmann (University of Dusseldorf) reported that in the liverwort *Sphaerocarpus* the size was nearer 37 μ m. Much less information is available about the function

of chloroplast DNA than about that of mitochondrial DNA. This is surprising in view of the primary role of chloroplasts in maintaining all living organisms. The view was expressed that this situation stems from the failure of too many departments of botany and biochemistry to promote interest in the biochemistry of plants; consequently far fewer biochemists study chloroplast development than study mitochondrial development. Several groups are currently preparing restriction fragments of chloroplast DNA, so some progress in mapping the chloroplast genome can be expected. One structural gene firmly established as located in this genome is that for the large subunit of Fraction I protein (ribulose-bis phosphate carboxylase). R. J. Ellis (University of Warwick) reported that this subunit was made *in vitro* by the free ribosomes of the chloroplast but not by the membrane-bound ribosomes. Isolated spinach chloroplasts will use light energy to incorporate ^3H -uridine into a discrete RNA species of molecular weight 2.7×10^6 ; M. R. Hartley (University of Warwick) and H. J. Bonert (University of Dusseldorf) reported hybridisation data which showed that this RNA species contains cistrons for both 16S and 23S chloroplast ribosomal RNA.

It is clear that most of the proteins of both mitochondria and chloroplasts are made on cytoplasmic ribosomes, and are then imported across the bounding membranes into the developing organelle. G. Schatz (University of Basle) suggested that the mechanism of specific protein uptake into these organelles is a major problem in cell biology, but no new data were presented at the meeting that shed any light on the process.

More and more promising

from David J. Miller

The International Particle Physics Conference was held in Palermo on July 23–28, 1975.

At last year's International Particle Physics conference in London, some theorists placed bets that "charm" would be discovered before this year's conference in Palermo. Charm is a suggested new quantum number, like the well-established "strangeness" number carried by hyperons and K-mesons. It had been incorporated into a very plausible scheme to explain the weak interactions, including the recently established neutral current effects, but no one had seen a "charmed" particle.

Now the Palermo conference has passed, but it is still not clear who has won the bets.

Remarkable things have been discovered during the year, the ψ (or J) and ψ' particles in particular. In the last few weeks a collaboration working at the DORIS electron-positron colliding-beam machine near Hamburg has reported a new intermediate state between the ψ and the ψ' . They claim that when their e^+e^- collision energy was 3.7 GeV (that is, when they were making ψ') they saw a number of events whose final state particles consisted of two fixed energy gamma rays and a ψ . The ψ was recognised when it decayed to a muon- or electron-pair with a mass of 3.1 GeV. The production of the two gamma rays is interpreted as a two-stage de-excitation cascade ($\psi' \rightarrow \text{new particle} + \text{gamma ray}$, followed by $\text{new particle} \rightarrow \psi + \text{gamma ray}$). Such cascades are encountered frequently in atomic or nuclear spectroscopy. Rumours from the SPEAR storage rings at Stanford, California suggest that similar intermediate levels have been observed there.

Charm lovers know exactly what they want these new objects to be. They say that the intermediate particle(s) are the p-wave states of "charmonium", that is, states with one unit of angular excitation. The ψ is supposed to be an s-wave ground state, and the ψ' is the first radial excitation. The states have been called charmonium by analogy with "positronium", a short lived atom-like structure formed from an electron and a positron. Charmonium should be made in a similar way from a charmed quark and a charmed antiquark. The only doubts one can have about this argument are that no one has yet proved that charm exists, and we haven't seen any quarks.

But progress has been made on both of these problems. The Stanford-Berkeley collaboration at SPEAR has now had time to collect e^+e^- annihilation data up to about twice the energy at which the ψ' is produced. They have seen one other bump, wider than the ψ and ψ' , centred at about 4.15 GeV, but they have also made a high-statistics run at a fixed rate collision energy of 4.8 GeV. This energy was chosen just because there seemed to be no specially interesting energy-dependent behaviour there. In the final states of this sample they have found evidence for another new phenomenon, the correlated production of muons and electrons. Combinations of a positive muon with a negative electron or of a negative muon with a positive electron have both been recognised, but there are no signs of combinations with the same charge for both the muon and the electron. The measured momenta

of the electrons and muons show that they do not carry 4.8 GeV of energy between them, so neutral particles must have escaped undetected.

Charm lovers are, once more, ready with an explanation. A pair of charmed mesons (real charmed particles, not charmonium) has been produced, they say. They must carry opposite charges, and they decay just like pions or K-mesons to muons or electrons, with neutrinos and perhaps neutral pions to take away the unseen energy. It is interesting that a very similar decay process for charmed particles could explain the neutrino-induced events with pairs of muons in the final state, reported at last year's conference by the Harvard-Pennsylvania-Wisconsin-Fermilab group, and confirmed this summer by the Caltech group, also working at Fermilab near Chicago. It is beginning to look as if the charm lovers may be right, but none of the evidence is really firm yet and physicists like to be sure before they pay out on a bet.

As for the problem of seeing quarks, theoretical physicists now tell us that we should not even try. Quarks exist, they say, but they can never be seen. They explain many of the properties of all strongly-interacting particles. They carry most of the mass, as well as the charge, strangeness and charm quantum numbers, but they can never be shaken free from the groupings of three quarks, or quark and antiquark, out of which particles are built. A group at the Massachusetts Institute of Technology has invented a model in which the quarks are permanently confined in "bags" about 10^{-15} m across. Other groups, including one at Cornell University, have models in which the quarks are tied on strings. Until recently these models were regarded as purely phenomenological; just rigged up to fit the data. But at Palermo, and afterwards at the Erice summer-school, near Palermo, it has become clear that fundamental field theories of elementary particles might provide very natural and attractive schemes in which quark-confinement could be achieved. This would be very exciting, since it could lead to a unified theory in which the strong, weak and electromagnetic interactions could be explained at the same time. At the moment some of the leaders in this effort, such as Coleman from Harvard or 't Hooft from Utrecht, are still proving beautiful theorems about two-dimensional space-time. K. Wilson of Cornell works in four dimensions, but his space and time form a quantised lattice. It may take a few years to get to the four-dimensional space-time continuum that physics actually happens in, but many theorists are very optimistic; although nobody is placing any bets.

review article

Did Chinese cosmology anticipate relativity?

John Gribbin*

Recent interest in historic novae and supernovae, stimulated by the discovery of pulsars, has shown the value of ancient Chinese astronomical observations. But the Chinese also have a long record of "cosmological" theorising; in some cases their old concepts regarding the Universe are remarkably similar to those developed in the West during the twentieth century.

ANY discussion of astronomy and cosmology in China in historic times must inevitably draw on Needham's work¹; it is therefore appropriate to begin such a discussion in what for an astronomer is the sobering light of the quotation from Franz Kühnert, made in Vienna in 1888 and which graces the title page of ref. 1: "Probably another reason why many Europeans consider the Chinese such barbarians is on account of the support they give to their Astronomers—people regarded by our cultivated Western mortals as completely useless. Yet there they rank with Heads of Departments and Secretaries of State. What frightful barbarism!"

Those "barbarians" were, however, thinking about the nature of the Universe, and producing some remarkably modern concepts, at least two thousand years ago. The earliest concept, of the heavens as a hemispherical dome closing over the Earth, and the slightly later concept of a celestial sphere surrounding the spherical Earth are familiar enough; it is possible that the earliest ideas can be traced back to Babylonian concepts of the world, and that the theories migrated both westward to Greece and eastward to China, where they developed independently. But it is particularly striking that one school of thought does not seem to have displaced the other in China. Followers of either theory could be found from the fourth century BC onwards, and there were also other schools. In terms of modern cosmology, the most striking of these ideas was not, it seems, the most popular some 2,000 years ago—but it was taught, and it is this system that most resembles modern ideas.

Infinite empty space

The Hsüan Yeh theory of the Universe was a later development and little is known about it before the Later Han (AD 25–AD 220). A little later, Ko Hung wrote (see ref. 1 p. 219): "The books of the Hsüan Yeh school were all lost, but Chi Mêng, one of the librarians, remembered what its masters before his time had taught concerning it. They said that the heavens were empty and void of substance . . .

"The sun the moon and the company of stars float freely in the empty space, moving or standing still. All are condensed vapour."

And in AD 336 Yü Hsi, the Chinese discoverer of the precession of the equinoxes, wrote: "I think that the heavens are

infinitely high, and that the space below the earth is unfathomably deep."

These ideas were some 1,300 years ahead of western thought (as far as there was any western thought about the nature of the Universe in the fourth century), and it is particularly interesting that western cosmology only broke the bounds imposed by the concept of the "crystal spheres" at about the time that reliable news of Chinese philosophy, mathematics and astronomy was beginning to reach the West. Not that the new ideas were broadcast as a great revelation by the Jesuits who visited China at the end of the sixteenth and beginning of the seventeenth centuries: they were held up to ridicule as examples of the stupidity of Chinese philosophers. Matteo Ricci listed several "absurdities" in Chinese astronomical thought in letters written in 1595 (see ref. 1 p. 438) including: "They say that there is only one sky and not ten skies; that it is empty and not solid. The stars are supposed to move in the void, instead of being attached to the firmament . . . Where we say there is air between the spheres, they affirm there is a void".

But these "absurdities" found a receptive audience in some western thinkers, at least. This was the time when western astronomy began to develop as a science, rather than as a branch of religion, and this development has led, 300 years later, to the present picture of an infinite Universe dotted with island galaxies. It is ironic that just when astronomy was making the crucial break with religion, orthodox western religious views were imposing a barrier on the further development of Chinese astronomy. The Jesuits persistently failed to broadcast new ideas such as those of Copernicus in China, and the centre of development of the science had definitely shifted to the West by the end of the seventeenth century. Even more ironically, a further 300 years of development in the west has only, it seems, brought cosmology to a level reached in China well before the time of the Jesuit mission. Even in the thirteenth century the concept of the plurality of worlds was well established, and Têngu Mu wrote (see ref. 1 p. 221): "Heaven and Earth are large, yet in the whole of empty space they are but as a small grain of rice . . . Empty space is like a kingdom and Heaven and Earth no more than a single individual person in that kingdom. How unreasonable it would be to suppose that besides the Heaven and Earth we can see there are no other heavens and no other earths."

To make that a modern statement, it is necessary only to replace "heavens" by "galaxies"; and if that concept had reached western cosmologists such as Thomas Wright, who

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published a remarkable theory of the structure of our Galaxy in 1750 (ref. 2), who knows what the consequences for the development of cosmology might have been.

Space and time

Perhaps the most important difference between the ancient Chinese thinkers and their western contemporaries was openness of mind. The Chinese were always, it seems, prepared to consider different theories of the Universe, rather than subscribing to

direction, above and below, is called yü." Thus, the Chinese expression yü-chou, commonly translated as "The Universe", means more literally "space-time"—a concept which only gained respectability in the West a few decades ago. This is not the only example of how the great philosophers of China seemed to foreshadow ideas now firmly attributed to Einstein. As early as 300 BC the Mohists taught (ref. 3 p. 221): "Movement in space requires duration . . . In movement, the motion of an observer must first be from what is nearer, and afterwards to what is further. The near and far constitute space. The earlier and later constitute duration. A person who moves in space requires duration." Although these ideas were so advanced, they have never, it seems, directly influenced the development of western ideas. That cannot be said of Chinese observational astronomy, however, which is even now providing vital fundamental information used by the most modern astronomers of all, the high energy astrophysicists.

Guest stars and Uhuru

Although it was the discovery of pulsars which first stirred renewed interest in Chinese records of guest stars, the most fruitful development recently has stemmed from observations of X-ray stars, made from the satellite Uhuru. The importance of the Chinese observations is that they provide accurate dates for the supernova explosions in which, it is generally believed, pulsars and X-ray stars are born. These sources are evolving so rapidly that information about their ages, and thus of their development over the past 2,000 yr or so, is of great importance.

The most famous Chinese guest star is, of course, the event recorded in June of AD 1054, which gave birth to the Crab Nebula, with its associated pulsar and X-ray source. But Cowley and MacConnell have identified six other X-ray stars with six guest stars dating from AD 1690, AD 1203, AD 902, AD 827, AD 722 and 48 BC (ref. 4). Some of these identifications are tentative, because the Chinese records are not always as accurate as modern astronomers would like (see Fig. 1). But if they are correct, they also provide a new insight into those ancient observations. Assuming that the X-ray stars have not moved far since they were created in the supernova explosions, the combination of modern positional information and the old records provides an accurate calibration of the Chinese unit of angular measure, and this in turn makes other old Chinese records more valuable.

There is still further interaction between modern astronomy and the Chinese records. Kiang, for example, has calculated the orbit of Halley's comet over the millenia⁵, and compared the calculated dates with dates of the appearance of comets (also referred to as guest stars) in the Chinese literature. That provides a new calibration of the Chinese calendars, and helps both historians and astronomers to make best use of the wealth of information available about China.

Perhaps there are still unsuspected ways in which this information might contribute to modern astronomy and cosmology; certainly the ideas are worthy of more than passing mention in teaching the subjects, and it would do no harm to students to draw their attention to such theories as the idea noted in a first century BC book (see ref. 1 p. 224): "The Earth is constantly in motion, never stopping, but men do not know it; they are like people sitting in a huge boat with the windows closed; the boat moves but those inside feel nothing."

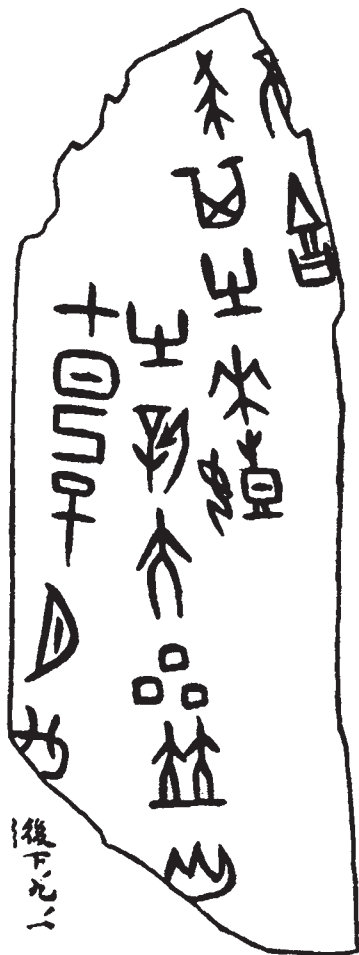


Fig. 1 Chinese oracle bone dating from about 1300 BC with the oldest existing written record of a nova (from Needham¹).

rigid doctrine. They also had a rather better grasp of the size of the Universe, both in space and time. Buddhist ideas of a cyclical Universe may have helped with the development of ideas about time; certainly the eighth century Chinese estimate of the time elapsed since the last "conjunction" (96,961,740 yr; see ref. 3 p. 45) was, while perhaps 100 times too small by modern estimates, still a lot more realistic than a European bishop's notorious eighteenth century estimate that the Earth was created on a certain day in 4004 BC, at six o'clock in the evening.

This grasp of space and time extended even to more abstract ideas. In a text of 120 BC quoted by Needham (ref. 3 p. 219) we find the following definition: "All the time that has passed from antiquity until now is called chou; all the space in every

¹ Needham, J., *Science and Civilisation in China*, 3 (Cambridge University Press, London, 1959).

² Wright, Thomas, *An Original Theory of the Universe* (Facsimile reprint of 1750 edition, Macdonald, London, 1971).

³ Needham, J., *The Grand Titration* (Allen and Unwin, London, 1969).

⁴ Cowley, A. P., and MacConnell, D. J., *Astrophys. Lett.*, 11, 217 (1972).

⁵ Kiang, T., *Mem. R. astr. Soc.*, 76, no 2 (1972).

articles

Similarity of genes *argF* and *argI*

Akihiko Kikuchi & Luigi Gorini

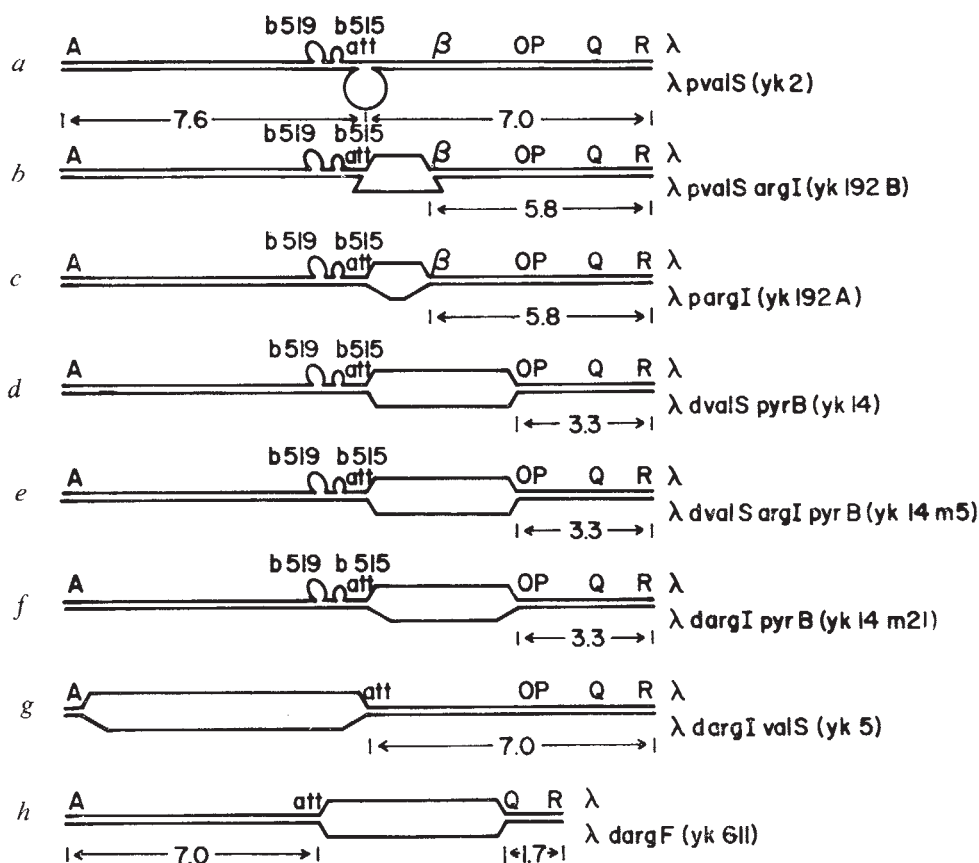
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Two genes, at different positions on the *Escherichia coli* genetic map but producing two enzymes which catalyse the same reaction, have a structure so similar that their DNAs anneal with each other in a heteroduplex mapping test.

COMPARED with eukaryotes, bacteria rarely have multiple copies of a gene. In some cases the duplicated gene is located very close to the original copy, usually in tandem as in the case of certain transfer RNAs¹. In other cases they are scattered without unique orientation, as in the case of the genes of rRNA, of which there are 5–7 copies². A need to increase gene dosage may account for these situations. The apparent lack of such a requirement makes the case of ornithine transcarbamylase

(OTC) more intriguing. This enzyme of the arginine synthetic pathway which converts ornithine to citrulline, is coded for by gene *argI* in all strains of *Escherichia coli* and *Salmonella* and is located at 85 min on the *E. coli* linkage map. In *E. coli* strain K12, however, an additional gene copy for the same enzyme, gene *argF*, exists and is located at 7.5 min of the *E. coli* map³. Why strain K12 should possess two OTC genes is not clear. The products of each of these two genes, *argI* and *argF*, have been studied biochemically. It has been found that four types of a trimeric enzyme molecule are formed by the random combination of two slightly different subunits coded for by each gene. The difference of each gene product is detected by ion exchange chromatography but not by size and shape of the subunits⁴. In addition the trimeric molecule formed by the *argI* product, is considerably more heat resistant than that consisting of the *argF* product (Glansdorff, personal com-

Fig. 1 Heteroduplex structure of λ specialised transducing phages. Heteroduplexes of DNA between λ c1857 S7 phage and each λ transducing phage are represented in the linear drawings. The double strand regions are represented proportionally while single strand loops are not. The double strand portion was measured by a map-measurer from a suitable enlargement and average values obtained from 10–20 heteroduplex molecules were normalised using *b519*, *b515* deletion loops as internal markers¹⁰. Total λ c1857 S7 phage DNA is 16.4 μ m long whereas λ y199 (carrying the two deletions *b519*, *b515*) is 14.6 μ m long using this type of measurement (error 5%). The distances indicated in the drawings are in μ m. All material including bacterial and phage strains and methods has already been described⁵ except for the heteroduplex mapping technique. After some failures encountered by using the recommended technique for the DNA of ϕ 80 phage particles⁸, we modified the technique for λ phage particles as follows: 1–5 μ l of the λ phage (10^{10} ml⁻¹) in CsCl solution ($\rho = 1.5$ g cm⁻³) is added to 5 μ l of 0.4 M EDTA-Na₃ and left at room temperature for 10 min. The phage solution was then diluted to a final volume of 25 μ l by double-distilled water and mixed with 25 μ l of another phage solution processed in the same way. The mixture of the two phages (50 μ l) is diluted to 400 μ l by adding 10 mM Tris-HCl pH 8.8, then denatured by 10 μ l of 5 N NaOH for 30 min at room temperature and then neutralised by 60 μ l of fresh 1:1 mixture of 2 M Tris-HCl pH 7.4 and 1.8 M HCl. The renaturation was started by the addition of 0.47 ml of 99% formamide and continued for 36 h at room temperature. During the renaturation a 60 μ l of Tris-HCl pH 8.8 was added to prevent the lowering of the pH.



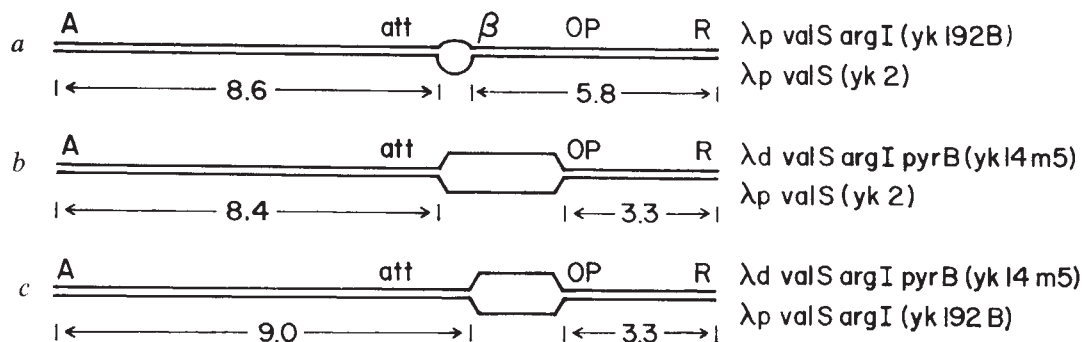


Fig. 2 Heteroduplex structure of λ specialised transducing phages carrying the *valS argI pyrB* region. The linear drawing represents heteroduplexes between each λ transducing phage. Details are described in the legend of Fig. 1.

munication). All other kinetic and affinity constants of the two enzymes are indistinguishable as well as the regulation pattern of the two operons, both being controlled to the same extent by the same repressor molecule, the *argR* product. Thus, the bulk of the two operons, *argF* and *argI*, which include promoters, operators and structural genes, might be so similar that the two DNA sequences could be able to anneal to a large extent if not completely.

Heteroduplex structure of the transducing phages

To demonstrate this hypothetical DNA homology, we first constructed several specialised transducing phages in the *argI* and *argF* regions of *E. coli* K12 chromosome. The structure of their heteroduplexes with the corresponding wild-type phages is presented in Fig. 1. In the *argI* region, because of two well defined markers, *pyrB* and *valS*, closely located at either side of the *argI* gene and 97% cotransducible with *argI* by phage P1, we could easily isolate λ specialised transducing phages of different sizes in that region of the chromosome. In addition to the previously isolated and described⁵ phages λ pvalS2 (yk2), λ dargI valS (yk5) and λ dvalS pyrB (yk14), we have obtained the following phages. λ pvalS argI (yk192B) and λ pargI (yk192A) were isolated from the HFT lysate obtained from strain RW420 (*proA/B argF lac*)[∇] (*gal attλ uvrB*)[∇] lysogenised with λ pvalS2 (yk2) phage using the *valS* gene homology. LFT transductants Arg⁺ were selected using the strain AD1 (*proA/B argF lac*)[∇] *argI*[∇] as recipient. On heat induction they produced HFT lysates of two types of phages, λ pvalS argI (yk192B) and λ pargI (yk192A). The lytic growth of yk192B always gives rise to a mixture of yk192B and yk192A (distinguishable by CsCl density gradient centrifugation) in different ratios depending on the conditions of the culture. The heteroduplex mapping shown in Fig. 1b and c, reveals that the λ pargI phage arises from λ pvalS argI by losing the *valS* portion. In a similar way the phage λ pvalS2 (Fig. 1a)

tends to lose *valS* and return to the parental phage λ y199 during lytic infection.

Phages λ dvalS argI pyrB (yk14m5) and λ dargI pyrB (yk14m21) (Fig. 1e and f) were selected from the lysate of strain BC22 *argI*⁺ *pyrB*[−], lysogenised with phage λ dvalS argI[∇] *pyrB*⁺ (yk14). In this way rare recombinants with the host chromosome appear which are Arg⁺. As seen in Fig. 1, there is no visible increase in DNA size in yk14m5, while there is a definite decrease in yk14m21 when they are compared with yk14 (Fig. 1d). The DNA length lost is of the order of 1.5 μ m, which corresponds to the size of *valS* insertion in λ pvalS2 phage (Fig. 1a). In the *argF* chromosomal region we obtained the phage λ dargF (yk611) (Fig. 1h) as already described⁵.

Physical mapping of the *argI* region

To demonstrate the exact location of *argI* genes in the transducing phages, we made a heteroduplex analysis between the transducing phages themselves. Figure 2 summarises the heteroduplex map of combinations of phages λ pvalS, λ pvalS argI, and λ dvalS argI pyrB. The gene order can be qualitatively recognised as *valS* → *argI* → *pyrB* starting from the attachment site, by the estimation of the length of double strand DNA and the size of single strand loop. For example, the double strand region at the left side of Figure 2a and b is the same and that at the right side of Fig. 2a is longer than that of Fig. 2b, indicating that *valS* gene is located at the left of *argI*. An analogous comparison between Fig. 2b and c permits the conclusion that *argI* is at the left side of *pyrB*. In addition, the short single strand loop in Fig. 2a should contain the *argI* gene, while the longer loop in Fig. 2b must contain *argI* and *pyrB* genes. The loop in Fig. 2c should contain at least the *pyrB* gene and probably something more at the right side. Figure 3 gives a clear view of the region because the heteroduplex between λ pargI and λ dvalS argI pyrB phages directly shows the *valS* size as an insertion loop at the attach-

Fig. 3 Electron micrograph of heteroduplex DNA between λ dvalS argI pyrB (yk14m5) and λ pargI (yk192A). A drawing is shown at the bottom in the same way described in Fig. 1. Black bars represent 1 μ m. The specimen for electron microscopy was prepared and examined as described⁵. We did not expect any heteroduplex between *l* and *l* strands (or *r* and *r* strands) because if any homology exists in the homologous strands of two genes oriented in opposite directions this will be too small to stabilise such a heteroduplex unless each strand is separated and then mixed with the corresponding strand⁶. Therefore, if we did not see any homology among a number of heteroduplexes (*l* × *r*) we concluded that either there is no homology between the corresponding parts of the gene or that the two genes in question are oriented in opposite directions.

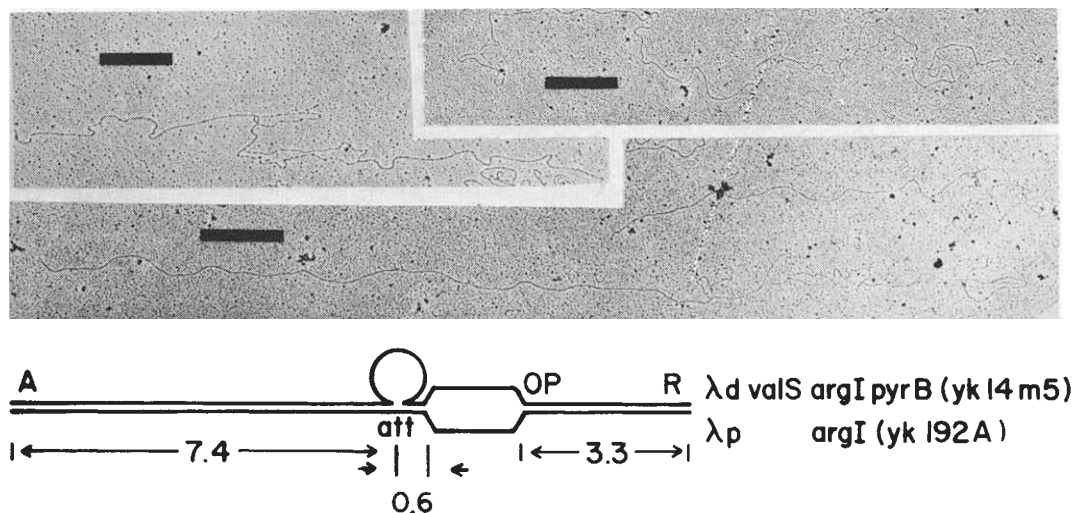
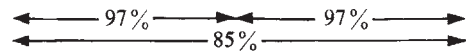
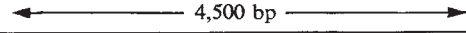


Table 1 The size and order of the *valS argI pyrB* gene

Genetic marker			
P1 cotransducing frequency			
DNA base pairs calculated from P1 transduction			
EM observation from Fig. 3			
EM observation for <i>argF/argI</i> duplex from Fig. 4			
DNA base pairs calculated from EM			
Molecular weight of the protein	110,000	35,000	33,000+17,000
DNA base pairs expected from the molecular weight	3,300 bp	1,050 bp	1,000 bp+500 bp

The following conversion indexes are used: 1 μ m DNA length=3,300 base pairs; 0.1 min map distance=4,500 bp; 1,000 bp correspond to a protein of molecular weight 33,000.

ment site, *argI* as a small successive double strand section and *pyrB* as a single stranded loop after *argI* at the right end.

The maximum size of each gene can be calculated as follows. The *valS* insertion is about 1.5 μ m which corresponds to about 5,000 base pairs (bp), the *valS* gene itself requiring only 3,000 bp. The *argI* portion is 0.6 μ m corresponding to 2,000 bp, but the *argI* gene itself should require only 1,000 bp. There is therefore a considerable space between *valS* and *argI* (about 1,000 bp at least) unaccounted for, which could indicate either another unknown gene or a mere spacer sequence. Finally *pyrB* has a length of 6,600 bp which is certainly too long for the *pyrB* gene. Since we do not have any marker after *pyrB* closer than *fdp* (which is separated by 15,000 bp calculated by P1 transduction frequency⁶), we could not obtain any phage for *pyrB* substitution enabling us to be more precise about the size of the *pyrB* gene. As it may be seen from Table 1, the overall distance of the three genes, *valS*, *argI* and *pyrB* is coincident with the P1 transducing frequency data.

argI, *argF* gene homology

From the physical mapping of the *argI* region we confirm the conclusion reached previously⁶ about the orientation of the *argI* gene in the λ phage. Since it is known that the operator of *argI* is located between *valS* and *argI* (ref. 7), we conclude that the *argI* gene is transcribed from left to right in the phage λ d*valS argI pyrB*. The same must be true for the phages λ p*valS argI* and λ p*argI* on the basis of the heteroduplex mapping results. By contrast, in the case of phage λ p*argI valS* (yk5), the orientation of *argI* must be the opposite since we could not detect homology between *valS argI* of λ p*valS argI* and *valS argI* of λ d*argI valS*. If there is considerable homology detectable by electron microscopy between *argF* and *argI* then we should see the *argF-argI* duplex in the heteroduplex either between λ d*argF* and λ d*valS argI pyrB* or between λ d*argF* and λ d*argI valS* (yk5). If there is no homology at all between the two OTC genes, we would not obtain a duplex in either combination. Figure 4a and b shows that duplex *argF-argI* is obtained

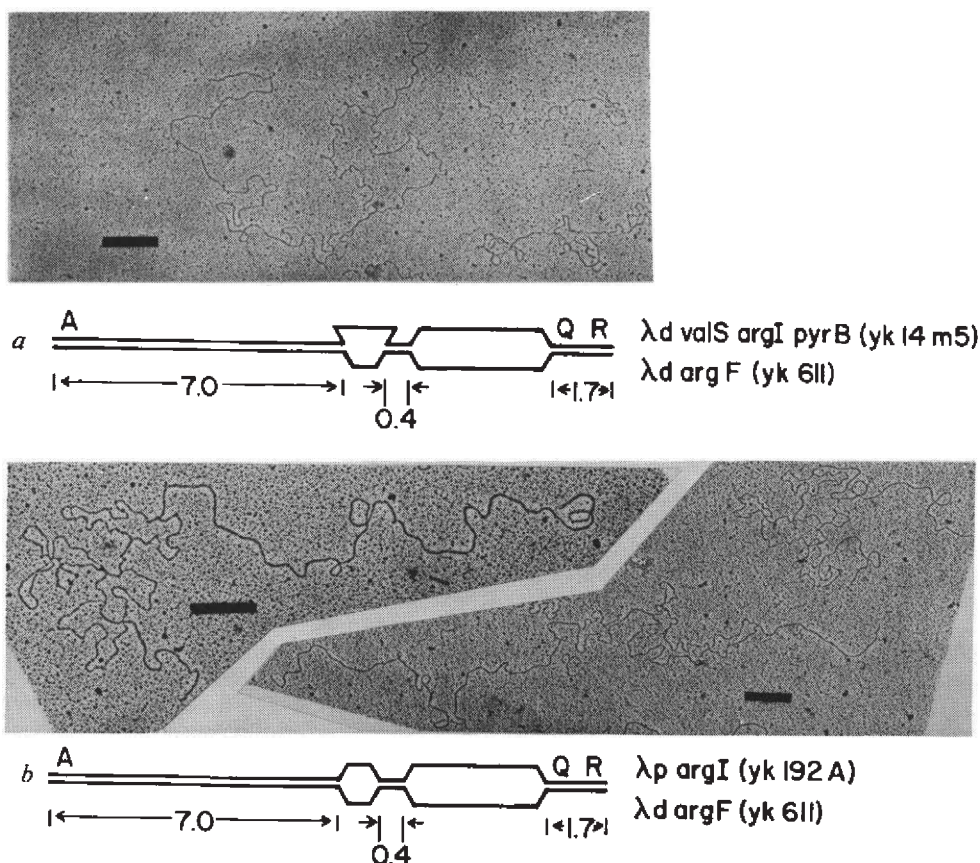
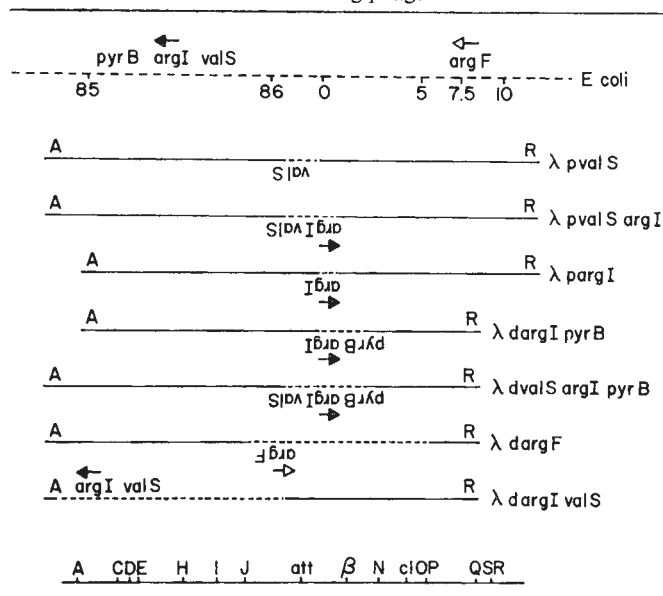


Fig. 4 Electron micrograph of heteroduplex DNA between λ d*argF* and λ d*valS argI pyrB* (a) or λ p*argI* (b). Black bars represent 0.5 μ m.

Table 2 Orientation of *E. coli* chromosomal markers on the λ transducing phage

The phage genes are represented at the bottom of the figure by a straight line with A end at the left and R end at the right. The *E. coli* chromosome is shown as a broken line and relative places of genes are shown on the top. The letter orders of each genetic marker are kept as it is in Taylor's map¹¹ to demonstrate the way in which the chromosomal portion was inserted into the λ genome. The assignment of gene order of λ pvalS, λ pvalS argI, λ pargI, λ dargI pyrB, λ dvalS argI pyrB and λ dargI valS are described in the text. The arrow on the *argI* represents the transcription direction from the operator mapping data of G. Jacoby⁷. The open arrow on the *argF* is determined from the fact that *argF* gene is annealed with the *argI* gene of λ dvalS argI pyrB phage.

with the combination λ dargF and λ dvalS argI pyrB or λ pargI. The location of duplex DNA between two single strand loops is exactly where *argI* gene is expected from the physical mapping data so that we believe that the duplex is between *argF* and *argI* although we could not confirm as yet whether the side opposite *argI* is indeed *argF* itself. The determined length of 0.4 μ m (1,200 bp) is in agreement with the size of 1,050 bp for

an OTC subunit (molecular weight, 35,000). We could not observe any heterogeneity within this duplex region although some minor base difference should be expected between the two OTC genes. Also we could not demonstrate any evidence for or against the operator-promoter homology. The size of 1,200 bp could accommodate such a region.

This result shows that the DNA sequences of *argI* and *argF* are extensively similar. Moreover, since the annealing extends for about 0.4 μ m which is the maximal length for the *argI* (or *argF*) gene, we can conclude that the homology extends over the entire length of the two genes. We do not expect that it should cover more because the surrounding genes at 85 and 7.5 min in the *E. coli* chromosome are completely different. Concerning orientation, our results only show that in this particular phage, yk611, the reading of *argF* is from left to right, the same as in phages λ pvalS argI pyrB or λ pvalS argI with which it anneals. The actual orientation of *argF* in the bacterial chromosome can only be assessed if we know in which way the *argF* has been inserted in the λ genome. According to considerations developed previously⁵ this insertion in yk611 is inverted in respect to the bacterial chromosome. If this conclusion is correct, then the *argF* gene orientation in the bacterial chromosome is counterclockwise as it is for the *argI* gene. These results are summarised in Table 2.

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Nucleotide sequence of a viral RNA fragment that binds to eukaryotic ribosomes

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The nucleotide sequence has been determined for the first 53 bases of brome mosaic virus RNA4, the monocistronic messenger for brome mosaic virus coat protein. The sequence includes the binding site for wheat embryo ribosomes. The 5'-terminal base is a modified guanosine attached to the penultimate base through a 5' p-p-p 5' link. The initiating AUG codon is only 10 nucleotides from the 5'-terminus. The 13 triplets following the AUG codon correspond to the known sequence of brome mosaic virus coat protein.

In the past few years, studies of nucleotide sequences of bacteriophage RNAs¹⁻⁸ have provided valuable information regarding the structure of initiation sites for protein synthesis in prokaryotes, but how ribosomes are able to select such initiator regions is still an open question. No sequences are known for the binding site of any eukaryotic RNA although recent progress with *in vitro* translating systems⁹ utilising such messengers has made sequence investigations both feasible and interesting. We report here the ribosome binding site of brome mosaic virus (BMV) RNA4 (Russian strain). BMV RNA4 is the smallest of the 4 RNAs contained in the virions of BMV, a multicomponent plant virus^{10,11} infectious to wheat. BMV RNA4 has been

shown to be an efficient, monocistronic messenger for BMV coat protein synthesis in a cell-free protein synthesising system derived from wheat embryo¹².

Isolation of fragments containing ribosome binding site

A number of workers⁴⁻⁸ have isolated ribosomal binding sites of bacteriophage RNAs, taking advantage of the fact that regions of RNA can be protected from nuclease digestion by their association with ribosomes. When we tried to isolate a ribosome-mRNA complex in this way with BMV RNA4 and wheat embryo ribosomes, we obtained a series of ribosome-protected fragments which appeared on polyacrylamide gels as closely stacked bands. Fingerprint analyses showed that these fragments contain initiation sequences, but it proved difficult to purify any one fragment in an amount sufficient for detailed analysis.

Rather than using ribosomes to protect binding sites from nuclease digestion, we found it preferable to use ribosomes to select mRNA fragments that contain binding sites. Wheat embryo ribosomes will bind certain BMV RNA4 fragments selectively. Among them are fragments that contain the codons for the N-terminal region of BMV coat protein, and these could be obtained in relatively pure form by choosing appropriate fractions from polyacrylamide gels.

The selection and purification of the binding site fragment were as follows. ³²P-labelled BMV RNA4 was digested under very mild conditions with ribonuclease T₁ and a part of the digest was subjected to polyacrylamide gel electrophoresis to examine the extent of degradation. The gel pattern is quite reproducible, indicating the introduction of cleavages at specific sites of the RNA. It contains one major band and many minor bands corresponding to fragments of various lengths. (Analysis of the major band showed that it is a fragment about 150 nucleotides long. It comes from the 3'-end of the RNA molecule and does not contain the ribosome binding site. Its characteristics will be reported elsewhere.) For ribosome binding, the partial digest was added to wheat embryo extracts under conditions that allow initiation of protein synthesis. Elongation of nascent peptide chains was inhibited by the addition of the antibiotic anisomycin¹³. Following incubation, the mixture of ribosomes and RNA fragments was centrifuged through a sucrose density gradient (Fig. 1). About 5% of the total radioactivity sedimented in the region of 80S, indicating a binding of some of the fragments to 80S ribosomes. Fractions containing the ribosome-fragments complex were combined and were extracted with phenol. RNA, consisting of unlabelled rRNA and radioactive RNA fragments, was precipitated from the aqueous layer with ethanol. The resuspended RNA was heated in 6 M urea at 60 °C, followed by a sucrose gradient sedimentation in the presence of urea. All the radioactive RNA was thus dissociated from the bulk of the rRNA and remained near the top of the gradient. Fractions containing radioactivity were pooled and the RNA was precipitated with ethanol. Figure 2a shows the gel electrophoresis pattern of this RNA. Its appearance is different from that of the unbound digest both in distribution and absolute amount of radioactivity. The pattern of Fig. 2a consists of two bands (bands I and II), corresponding to RNA fragments of relatively small size, along with bands corresponding to larger fragments of a variety of lengths. Sequence analysis (see below) showed that the fragments of bands I and II were indeed ribosome binding sites, and the most prominent bands (for example, bands *i*, *ii* and *iii*) corresponding to the larger fragments also contained all of the binding site oligonucleotides. Since the fragments corresponding to bands I and II are short, exist in high molar amount and can be obtained readily in pure form, they were chosen for detailed sequence studies. It may be mentioned here, for example, that although band *iii* exists in Fig. 2a in almost the same radioactive amount as band I, its molar amount is an order of magnitude smaller because it contains a factor of 10 more bases.

For some experiments, fragments were eluted from crushed,

fractionated gels and were studied without further purification. We found that band I and band II materials could, however, be obtained in purer form if the separation of the RNA fragments from rRNA was carried out in two stages.

The RNA obtained from the phenol extraction procedure was first fractionated on a sucrose density gradient without urea treatment. After centrifugation, about half of the radioactivity remained near the top of the gradient while the other half sedimented with the rRNA. Gel electrophoretic analyses showed that fractions from the top of the gradient contained mostly the larger fragments exhibited in Fig. 2a but relatively small amounts of band I and band II material. The 18S RNA fractions contained band I and very little else. The 28S RNA fractions contained band I and band II materials in roughly equal amounts, together with some heterogeneous larger material. Generally, we combined the 18S and 28S fractions, subjected them to gel electrophoresis, and eluted bands I and II for purposes of sequence analysis. Figure 2b shows the gel electrophoresis pattern of RNA fragments obtained in this way. About 0.2% of the radioactivity used for binding was recovered in

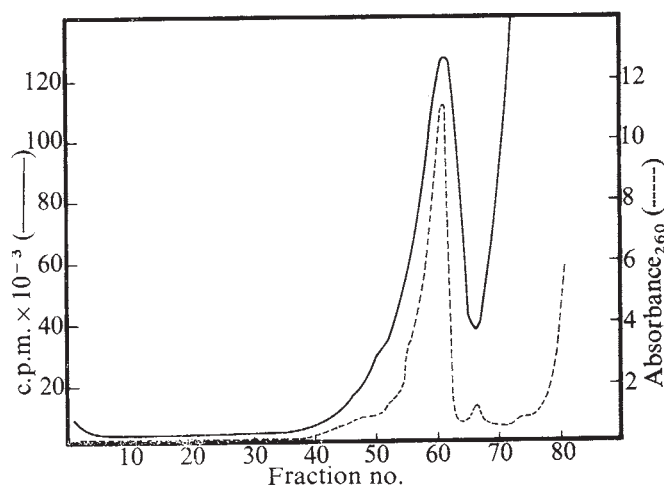


Fig. 1 Binding of the RNA4 fragments to ribosomes. BMV RNA labelled with ³²P was prepared as described previously^{12,29}. Samples (120 µg; specific activity 0.5 µCi per µg RNA) were digested with 0.12 µg of RNase T₁ (enzyme: substrate = 1:1,000) for 15–20 min at 0 °C in 100 µl of buffer consisting of 0.1 M NaCl, 0.01 M magnesium acetate and 0.025 M Tris-HCl (pH 7.5). After incubation, the reaction mixture was diluted to 0.5 ml with the above buffer, extracted with an equal volume of phenol at 0–4 °C and precipitated twice with 2 volumes of ethanol. The final precipitate was dissolved in 50 µl of distilled water. Binding of the RNA fragments was carried out in a wheat embryo cell-free extract. A procedure, modified from Davies and Kaesberg³⁰, was used to prepare a cell-free extract. After homogenisation the 23,000g supernatant was made 4 mM in magnesium acetate and preincubated with 20 mM Tris-acetate, 1 mM ATP, 0.06 mM GTP, 10 mM creatine phosphate, 75 µg ml⁻¹ creatine phosphokinase (from Calbiochem, containing 58% protein) and 1 mM dithiothreitol at 30 °C for 20 min. Ten millilitres of the preincubated extract was then passed through a Sephadex G-25 column (2 × 41 cm) equilibrated with 10 mM Tris-acetate, pH 7.6, 95 mM potassium acetate, 1 mM magnesium acetate and 1 mM dithiothreitol. Fractions with A₂₆₀ between 40 and 220 were pooled and stored at –90 °C in small aliquots. Binding was performed in a 2 ml reaction mixture containing 100 µg (5 × 10⁷ c.p.m.) of the RNA partial digest, 1 ml S23, 20 mM HEPES, pH 7.6, 2.5 mM ATP, 0.38 mM GTP, 10 mM creatine phosphate, 250 µg creatine phosphokinase, 0.04 mM each of the 20 amino acids and 25 µg of anisomycin. The reaction mixture was made 4 mM in magnesium acetate and 95 mM potassium acetate and incubated at 30 °C for 20 min. The mixture was quickly cooled on ice and applied to three 10–40% sucrose density gradients prepared in 50 mM Tris, pH 7.6, 50 mM KCl and 4 mM magnesium acetate. The gradients were centrifuged at 4 °C in a Spinco SW27 rotor for 4 h at 27,000 r.p.m. Half millilitre fractions of the gradients were collected manually by puncturing the bottom of the tube and counted. The absorbance at 260 nm was measured in a Cary spectrophotometer. Radioactivity was measured as Cerenkov radiation in a Beckman LS300 scintillation counter.

bands I and II. The band I and band II fragments can be rebound to ribosomes but we have not found conditions to bind them to purified rRNA.

Sequence analysis of the initiator fragments

T_1 and pancreatic RNase fingerprints of the RNA fragments isolated from band I and band II are shown in Fig. 3. The yields and the molar ratios of the oligonucleotides were very consistent from one experiment to another, as would be expected for reproducible binding of a specific BMV RNA4 fragment. The fingerprints of band II RNA contain all the oligonucleotides of those of band I, suggesting that band I contains a BMV RNA4 fragment consisting of a part of that of the band II fragment. The primary sequences of most of the oligonucleotides in the fingerprints were determined by standard methods¹⁴⁻¹⁶. The sequence of the large oligonucleotide UAUUAAUAAUG at the top of the T_1 fingerprint was determined by two dimensional homochromatography after partial spleen¹⁶ and partial pancreatic RNase digestion followed by base composition analysis of the products. The sequence of the T_1 oligonucleotide XG (corresponding to pancreatic oligonucleotide XGU) requires special comment. Its structure has been determined in collaboration with Dr Fumio Harada. It was found to be $m^7G^{5'}ppp^{5'}Gp$. The evidence for this structure will be described in detail elsewhere but can be summarised as follows: (1) the oligonucleotide XG is completely resistant to digestion with T_2 RNase and pancreatic RNase; (2) treatment with alkaline phosphatase released only 25% of its radioactivity as inorganic phosphate; (3) limited digestion with snake venom phosphodiesterase released 7-methylguanosine-5'-phosphate and $ppGp$ by the scission $m^7G^{5'}p/pp^{5'}Gp$. The products were identified by two dimensional thin layer chromatography¹⁷ and by coelectrophoresis with authentic markers; (4) complete digestion with snake venom phosphodiesterase converted the oligonucleotide into the products: pm^7G , pGp and P_i , that is, by the scission $m^7G^{5'}p/p^{5'}Gp$. This indicates that the m^7G has a free 3' hydroxyl and is linked through its 5'-phosphate; (5) nucleotide pyrophosphatase, which can cleave pyrophosphate linkages, cleaved 7-methylguanosine-5'-phosphate from the oligonucleotide, giving the same products as above.

This oligomer is the only T_1 product having a 5'-phosphate and it has been detected as such in the T_1 fingerprint of the BMV RNA4 molecule itself. Periodate oxidation and β -elimination of BMV RNA4 removed the m^7G , giving rise to a new T_1 product $pppGp$. All of this evidence indicates that m^7G has a 3' hydroxyl group and that the structure $m^7G^{5'}ppp^{5'}Gp$ must be present at the 5'-end of the RNA molecule. Thus, the 5'-end of the ribosome-bound fragments comes from the 5'-end of RNA4.

The complete nucleotide sequence of the band II RNA is shown in Fig. 4 as achieved from overlapping sequences in T_1 and pancreatic oligonucleotides, partial T_1 RNase digestion and base analysis of the products. Band I contains the first 22 nucleotides of band II. We have determined the sequence of the first 10 amino acids of *in vitro* synthesised BMV coat protein (Russian strain) by use of an Edman Begg automatic sequencer and in the same manner described for the determination of other parts of the BMV coat protein sequence¹⁸. The sequence is Ser-Thr-Ser-Gly-Thr-Gly-Lys-Met-Thr-Arg. This sequence is in agreement with that reported for the first 25 amino acids of the wild-type BMV coat protein¹⁹. The sequence of the first 14 amino acids, together with the nucleotide sequence of band II, is shown in Fig. 4. According to the genetic code, the protein sequence corresponds precisely to the nucleotide sequence following the initiation codon AUG at position 10.

Features of the sequence

In the preceding section we have presented the nucleotide sequence of the portion of BMV RNA4 specifically recognised by wheat embryo ribosomes. Its most distinctive feature is the location of the initiation codon only 10 nucleotides from the 5'-terminus. This is in marked contrast to the situation for the

sequences of bacteriophage RNAs. The initiation codons of the first cistron (A-protein cistron) of phage R17 and phage MS2 RNA are located 130 nucleotides from the 5'-terminus^{3,4,20} and those of Q β RNA are 61 nucleotides from the

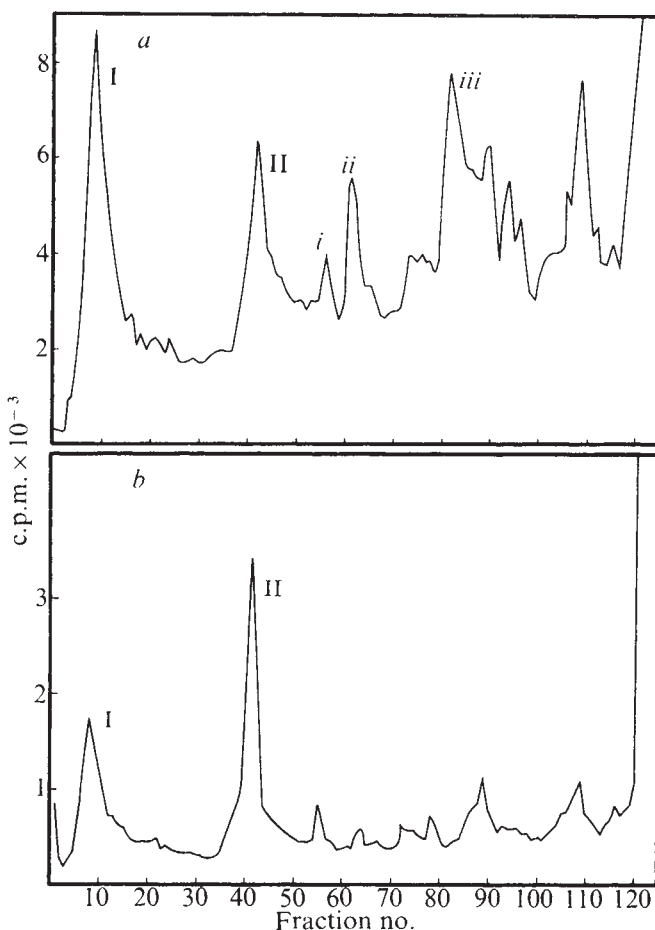


Fig. 2 Gel electrophoresis of RNA fragments isolated from the ribosome binding complex. The fractions from the 80S ribosome region in Fig. 1 were pooled and precipitated with two volumes of ethanol. The pellet was resuspended in 1 ml of 0.2 M Tris-HCl (pH 7.4), 0.2% sodium dodecyl sulphate and extracted with an equal volume of phenol. The RNA was then precipitated with ethanol. *a*, The precipitate was dissolved in 0.5 ml of a suspension buffer containing 0.02 M NaCl, 0.005 M Tris-HCl (pH 8.3) and 6 M urea and the solution was heated at 60 °C for 10 min to dissociate ^{32}P -labelled RNA from the unlabelled RNA. The solution was cooled to 0 °C and diluted with an equal volume of the suspension buffer without urea. The solution was then applied to a 5–20% sucrose gradient prepared in the suspension buffer containing 3 M urea. The gradient was centrifuged at 4 °C in a Spinco SW27 rotor for 20 h at 26,000 r.p.m. The fractionation of the gradient and the measurement of the absorbance and the radioactivity of each fraction was done as described in Fig. 1. The first 10 fractions from the top were pooled, the RNA was precipitated with ethanol, and dissolved in 50 μ l of the TEB buffer of Peacock and Dingman²¹ and 5 μ l of a solution containing 50% sucrose and 0.1% bromophenol blue was added to it. The sample was then loaded on a 10% polyacrylamide gel and subjected to electrophoresis at a constant current of 3 mA per gel for 2.5 h at room temperature. The gel was crushed in an automatic Gilson gel crusher which crushed gels into fractions corresponding to gel slices 1 mm thick. Electrophoresis was from right to left. *b*, After phenol extraction and ethanol precipitation, the ribosome-bound RNA was dissolved in 0.5 ml of 0.2 M NaCl, 0.05 M Tris-HCl (pH 8.0), and then applied to a 5–20% sucrose gradient made in the same buffer. Centrifugation and fractionation of the gradients were done as described above. The RNA from the 18S and 28S regions was pooled, combined and precipitated with ethanol. The precipitate was dissolved in 0.5 ml of the suspension buffer containing 6 M urea, heated at 60 °C for 10 min and then subjected to sucrose gradient containing 3 M urea as described in (*a*). The ^{32}P -labelled RNA was isolated and subjected to gel electrophoresis.

5'-terminus¹. BMV RNA4 has an estimated chain length of approximately 900 bases. Roughly 600 of these bases are assignable to the coat protein cistron¹². This suggests that one-third of the BMV RNA4 sequence, adjacent to the 3'-end, has an unknown function other than that of encoding a protein sequence.

BMV RNA4 is unusual in another respect. Although it is a monocistronic messenger for the BMV coat protein, this RNA is not required for infectivity. BMV RNAs 1, 2 and 3 are needed for infectivity; RNA4 is regenerated in the infection process and is present in progeny virions. The sequence of RNA4 is a part of the sequence of RNA3 and complementation studies verify that RNA3 contains the coat protein cistron. The coat protein cistron, however, is translated *in vitro* to only a small extent from RNA3, although a second protein, designated protein 3a, is well translated. We infer that *in vivo*, some

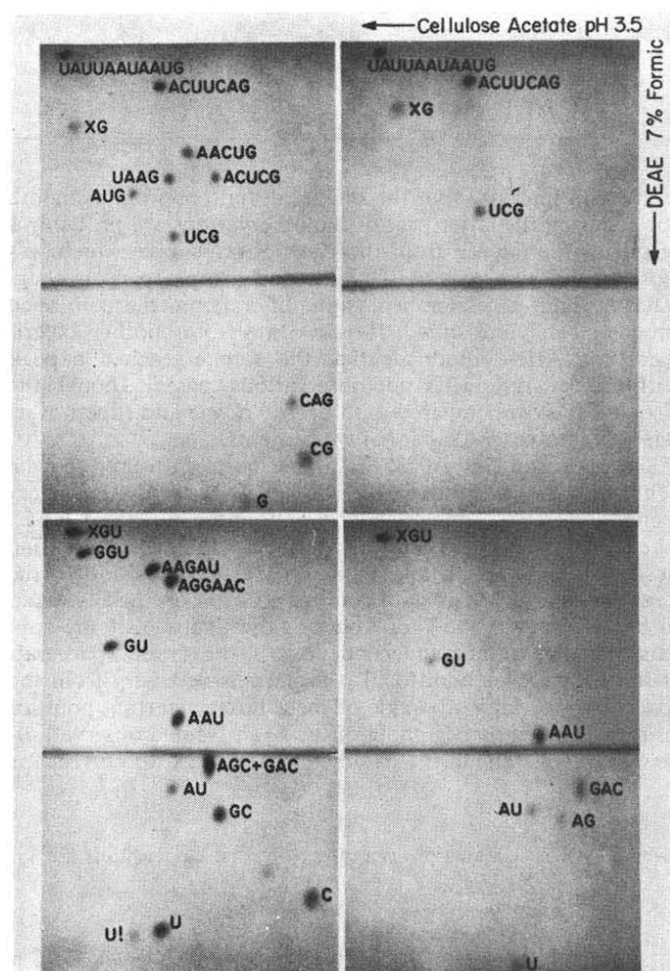


Fig. 3 RNase fingerprints of ribosome binding fragments of BMV RNA. *a*, T₁ RNase digest of band II; *b*, T₁ RNase digest of band I; *c*, pancreatic RNase digest of band II; *d*, pancreatic RNase digest of band I. Fractions corresponding to band I and band II shown in Fig. 2*b* were pooled and RNA was eluted by shaking the gel particles in 2 ml of 0.25 M NaCl for 3 h at room temperature. Gel particles were removed by Millipore filters and 50 µg of unlabelled yeast RNA was added to the filtrate. RNA was recovered by ethanol precipitation. After one additional ethanol precipitation, each sample was divided into two parts and subjected to fingerprint analysis following standard procedures¹⁴. For determining the sequence UAUUAUAUG, the T₁ oligomer was divided in to two parts and subjected to limited digestion with 2 µl of a solution of spleen phosphodiesterase (5 mg ml⁻¹; 2 min at room temperature) and pancreatic RNase (50 µg ml⁻¹ containing 5 mg ml⁻¹ unlabelled yeast RNA; 15 min at 4 °C). The products were separated by two-dimensional homochromatography and identified by complete digestion with pancreatic RNase. XG is m⁷G 5' p-p-p 5' Gp. Oligonucleotide AG in the pancreatic fingerprint of band I (*d*) comes from the 3'-end of the fragment

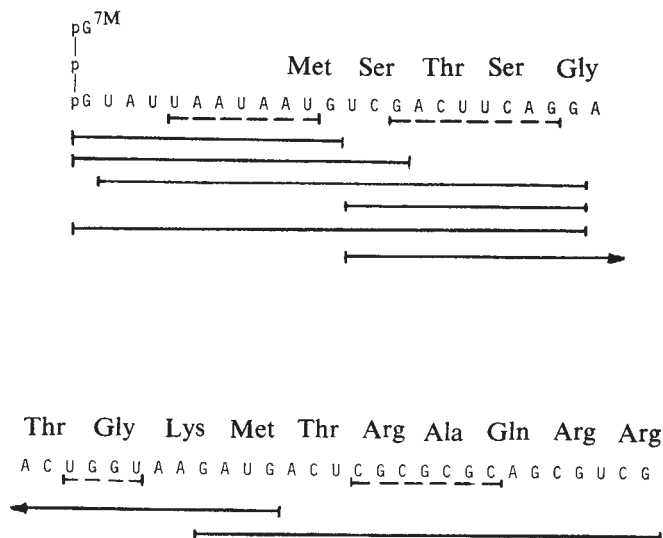


Fig. 4 Nucleotide sequence of the ribosome-binding fragment of band II shown with the N-terminal amino acid sequence of the coat protein. T₁ partial products which helped to deduce the sequence are shown by solid lines. These were obtained by a limited digestion of the band I and band II material with an enzyme: substrate ratio of 1:500 for 15 min at 4 °C. The products were fractionated in two dimensions by homochromatography and identified by fingerprint analysis after complete digestion with T₁ RNase. Palindromes are shown by dotted lines.

of RNA3 is processed—presumably by nucleolytic cleavage, replication and 5'-terminal modification, to yield RNA4. Thus, a pre-translational cleavage is necessary for expression of the coat protein cistron. It may be mentioned that RNA3 also has a m⁷G 5' p-p-p 5' Gp terminus and it is probably implicated in the effective translation of protein 3a.

The presence of 7-methylguanosine and the pyrophosphate linkage has been recently reported to occur in viral messenger RNA from reovirus²¹, vaccinia virus^{22,23} and cytoplasmic polyhedrosis virus²⁴. It is also present in Novikoff hepatoma cell nuclear RNA²⁵, and the reovirus genome RNA²⁶. Our fingerprint analysis of BMV RNA4 shows that about 50 to 75 % of it contains this structure whereas the BMV RNA4 binding fragments contain a quantitative amount. This difference could mean that some of BMV RNA4 lacks part or all of the distinctive 5'-terminus. Possibly ribosomes select those fragments containing it. We find, however, that the oligomer m⁷G 5' p-p-p 5' Gp does not bind significantly to wheat embryo ribosomes so that this structure alone, cannot be responsible for the binding of ribosomes. Our binding results show that the fragment of band I can bind ribosomes as efficiently as the band II fragment. This implies that the ribosome binding signal resides within the first 22 nucleotides. The binding site sequence does not form a stable secondary structure as evaluated according to Tinoco *et al.*²⁷. The association of the initiation fragments with rRNA implies a sequence complementarity between messenger and rRNA²⁸. We suggest that the unique 5'-terminus, the short sequence of As and Us, and the AUG codon constitute a simple and efficient ribosome recognition and binding site.

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letters to nature

Observations of a transient X-ray source with a period of 104 s

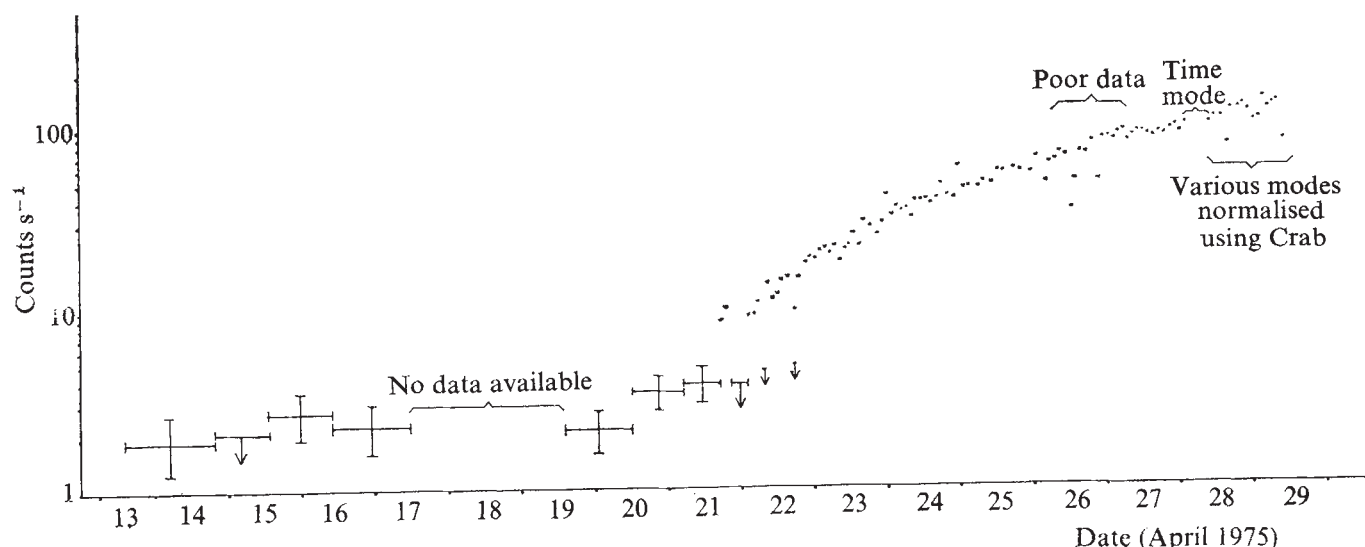
DURING routine observations of the Crab Nebula by Ariel V, experiment A, the rotation modulation collimator (RMC), discovered a new X-ray source A0535+26. The source was just detectable during the first observation on April 13, and during the 16 d of observation was seen to brighten to nearly twice the Crab's intensity (in the 3–7 keV range). The source varies periodically, with a period of 104 s, and has a very flat spectrum in the 3–7 keV range.

Figure 1 shows the observed count rate from A0535+26 as a function of time in units of counts s^{-1} in the 3–7 keV range corrected for the collimator response. Intensities before April 21 were obtained by overlaying several orbits of data together. An approximate ratio between Uhuru counts and the RMC counts is 13:1. The source was definitely present at a low irregular level several days before the main increase started, and it returned to a low level or turned off for short periods during the early stages of increase. This behaviour is reminiscent of that shown by A1118–61 (refs 1, 2) which returned to a low level twice in the period immediately before the main

increase in intensity. But the smaller deviations from a smooth curve during the latter part of the observations are probably a result of interference from the Crab X-ray source which was close to the spin axis at the time and are probably not significant. Thus the later behaviour differs from the continued irregular behaviour of A1118–61. Data from another experiment³ on Ariel V indicate that the source reached a peak within a few days after our observations ceased. Though the rise time is slow compared with optical novae and supernovae, it is comparable with other X-ray transients.

The position of A0535+26 has been measured to be $05^h35^m14.7^s \pm 26^{\circ}16'52'' \pm 45''$ (1950.0) (90% confidence) which differs from our previously announced position⁴ by $2'$. The RMC determines positions relative to the spin axis pointing position of the spacecraft. Using the relative position of the Crab Nebula from our data, and its position on the sky taken to be $15''$ NW of the radio pulsar⁵, the attitude of the spin axis was determined independently of the crude spacecraft attitude data. One additional parameter is necessary from the spacecraft, the azimuth angle of the arbitrary starting point of its rotation. At the beginning of the Crab Nebula observations new spacecraft alignment parameters were used to correct for

Fig. 1 Light curve of A0535+26. Bars indicate periods of integration (where these exceed one orbit) and $\pm 1\sigma$ errors. Upper limits are 3σ .



previously large ($\sim 0.5^\circ$) systematic errors in the spacecraft attitude solutions. But it was not until after the Crab Nebula observations (in the Cygnus region using Cyg X-1 and Cyg X-3 as benchmarks) that it was found that the new alignment parameters caused a change in azimuth angle (zero sector angle) of $20'$. This in turn caused a change in right ascension at the position of A0535+26 of $2'$.

During four orbits on April 28, 1975, data were collected in the experiment's "time" mode. In this mode data are integrated for 32 s and stored. Each orbit yielded about 45 min of useful data, which were collected starting at 4.21695, 5.80538, 7.390116 and 8.99238 h UT. The average count in 32 s was 6,000, made up by a 40% contribution due to A0535+26, and 30% each due to the Crab and to total particle and diffuse X-ray background in the 17° FWHM field of view. The sum of the power spectra for all four orbits of time mode data is shown in Fig. 2. This power spectrum differs from that expected from a constant signal with statistical noise fluctuations in three ways: (1) an excess low frequency component; (2) excess power at all frequencies as compared to expected statistical fluctuations; and (3) marked sinusoidal features at 0.960×10^{-2} Hz (104.1 s) and 1.20×10^{-2} Hz (83.3 s).

The low frequency component is a result of a combination of slow drift in the intensity of the source and background, and

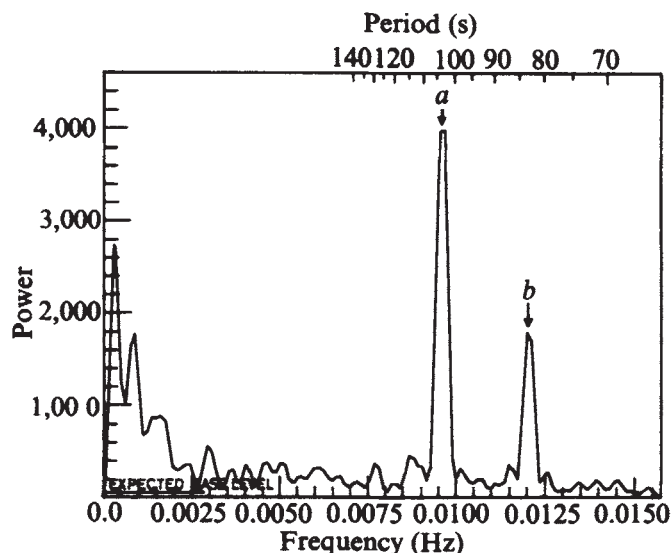


Fig. 2 Sum of power spectra of the four orbits in TIME mode. Only the mean has been removed. The height of the 104.1-s peak corresponds to a 25% modulation of the source, and its width is that expected for a pure sine wave. *a*, 104.1 s; *b*, 83.3 s.

Table 1 Possible periods which would be aliased to 104.14 s with a sampling time of 32 s

Period (s)	Frequency (Hz)	Attenuation factor
104.14	0.96×10^{-2}	0.852
46.1	2.17	0.376
24.4	4.09	-0.201
18.9	5.29	-0.154
13.9	7.21	0.112
11.9	8.42	0.098
9.7	10.34	-0.078
8.7	11.54	-0.073
7.4	13.46	0.063
6.8	14.66	0.054

The attenuation factor caused by the finite sampling is also shown.

to the finite sampling time (45 min). Removing a quadratic trend in the data, from each orbit independently, reduces the low frequency component. The excess power over all frequencies indicates that the source is randomly fluctuating over all periods between several seconds to minutes at least.

The periodic feature at 0.960×10^{-2} Hz must be accepted as real, and not an artefact of some type of beating phenomenon between the modulation caused by the rotating grids and the sampling frequency. Since the rotation rate of the spacecraft is nominally 10 r.p.m. the 32-s integration time represents an integration over more than 10 half-rotations of the RMC. Analysis of time mode data from the region of the galactic centre and Cyg X-1 taken before and after the observations reported here do not show any periodic components, nor any comparable power over the measured range of frequencies.

But it is not necessary that the 104.1 and 83.3 s periods are the true incident periods. Because of the integration time of 32 s any frequency above the Nyquist frequency $1/64 \text{ s}^{-1}$ would be aliased to a measured period greater than 64 s. Indeed the 83.3 s period is explained as the 52 s harmonic of the 104 s fundamental aliased to 83.3 s. Furthermore, the 104 and 52 s periodicities could also be aliases of shorter period oscillations. Table 1 lists the first 10 possible higher periods which could be aliased to 104 s with a sampling rate of 32 s. The second harmonic of any of these frequencies would be aliased to produce an apparent period of 83.3 s.

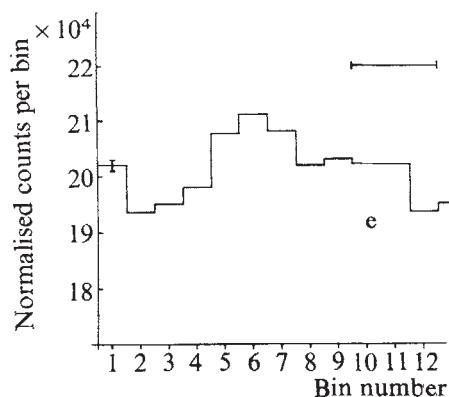
But there is a means of differentiating between some of the possible different frequencies. Since data are integrated for 32 s

the observed periodic signal will have been attenuated by a factor depending on its frequency. The attenuation factors for the possible fundamental frequencies are shown in Table 1. The observed modulation of the signal was 25% of the mean. The shorter periods (24.4 s and less) are precluded because they would imply a modulation depth of the incident signal greater than 100%.

An epoch-folding analysis yields the best estimate for the period of $104.14 \pm 0.16 \text{ s}$ (1σ). Figure 3 shows the observed light curve for the 104.14 s periodicity, folded into 10 bins for all 340 32-s samples over the four orbits. The third harmonic, if it exists, is aliased near the DC level and is masked by any slow trends in the data. The fourth harmonic is too weak to be seen above the noise. Because of the relatively long sampling time, and thus the low sensitivity to the higher harmonics, no attempt has been made to deconvolve the measured light curve with the instrumental response.

The light curve of the source (Fig. 1) shows no sign eclipses; the time mode data are insufficient to measure change in period of the dwarf in its orbit.

Fig. 3 The light curve of the 104.14-s period folded in bins, shown over two cycles. The error shown is statistical, 32-s sample time.



Crude three channel energy resolution (3–7.5 keV) shows that around its peak the spectrum of this source is very similar to the transient A1118–61 (ref. 2). There are indications that during the growth phase the absorption increased to this value from a low level.

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Hard X-ray measurements of nova A0535+26 in Taurus

MEASUREMENTS of high energy X rays in the region of the Crab Nebula were made with the Imperial College detector on Ariel V between April 13 and April 29, 1975. During that time the X-ray nova A0535+26 was first detected and observed to rise to its maximum intensity^{1,2}. At 30 keV this nova reached an intensity considerably brighter than the Crab Nebula, making it the brightest known source in the sky.

Our experiment uses an actively shielded crystal scintillator, and operated in the energy range 26 keV–1.2 MeV, making

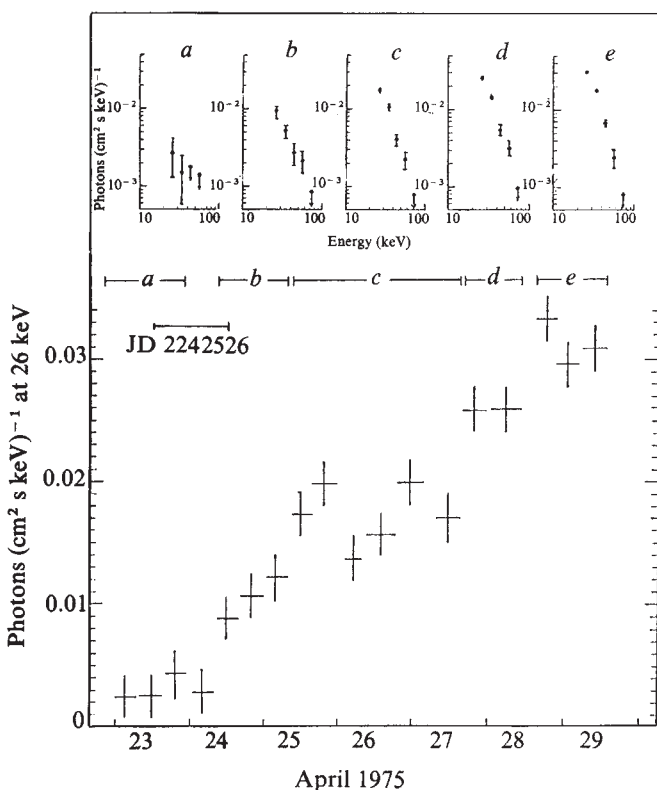


Fig. 1 Light curve of nova A0535+26 obtained from channel 1 of the high energy experiment, with an 8-h time resolution. Data have been normalised to 26 keV. Errors are $\pm 1\sigma$ counting statistics. *a-e*, Five spectra obtained during this period; the data collection period for each spectrum is indicated.

spectral measurements in 16 logarithmically spaced energy channels. It has a sensitive area of 8 cm² and an opening angle of 8° FWHM which is offset from the spacecraft spin axis by 3°. A source a few degrees from the spin axis direction can be detected by determining the modulation in the counting rate accumulated in four equal angle spin sectors. Since a spurious modulation is induced in the apparatus by cosmic rays, a separate measurement is required to subtract this background. This is normally achieved by making observations at offsets on either side of the source; however, in the case of the nova discussed here, data for background subtraction were taken from a period before the nova had reached a significant intensity as measured by the low energy experiments.

For most of the observations reported here, the spin axis was pointing to within 0.5° of the Crab Nebula which does not give any significant modulation for this offset angle. Data for

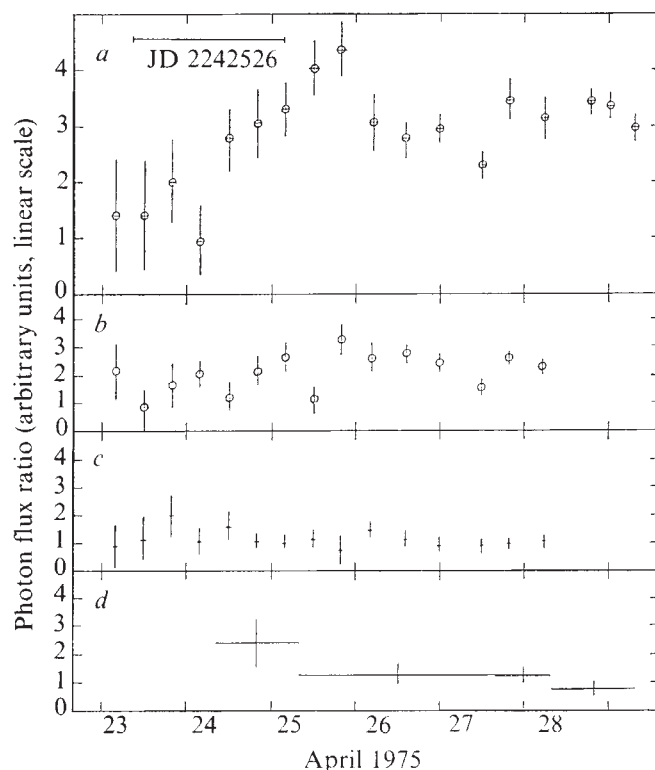


Fig. 2 Photon flux ratios at various energies plotted to indicate any spectral changes during the rise of the event. *a-c*, Ratio of the fluxes observed in channels 1–3, respectively, to those measured by the RMC experiment, *d*, the ratio of the flux in channel 4 to that in channel 1 of the high energy experiment. Error bars indicate $\pm 1\sigma$ in counting statistics. Energy intervals: RMC experiment, 2.95–7.60 keV; our experiment: channel 1, 26–33 keV; channel 2, 33–43 keV; channel 3, 43–56 keV; channel 4, 56–73 keV.

background subtraction were obtained on April 20, 1975, and it is assumed that the nova source had not reached a detectable level for this experiment on that day. The position of the nova¹ places it 4° from the spin axis which happens to be the offset giving maximum modulation and therefore maximum sensitivity for this experiment. For the last day of the observation the spin axis was offset 1.4° from the Crab Nebula towards the nova and corrections had to be applied, first for the signal now produced by the Crab Nebula and second for the slight reduction in efficiency because of the reduced offset from the nova. Data from previous measurements on the Crab Nebula were used to make these corrections.

Figure 1 shows the light curve obtained from the lowest energy channel with an 8-h time resolution starting when the first statistically significant signal was observed. The flux has

been corrected to the lower threshold of this channel (26 keV). The phase of the spin modulation for this data corresponds to the stated position of the nova; any spurious signal originating from the Crab Nebula would change this phase, which is kept as a free parameter in the analysis.

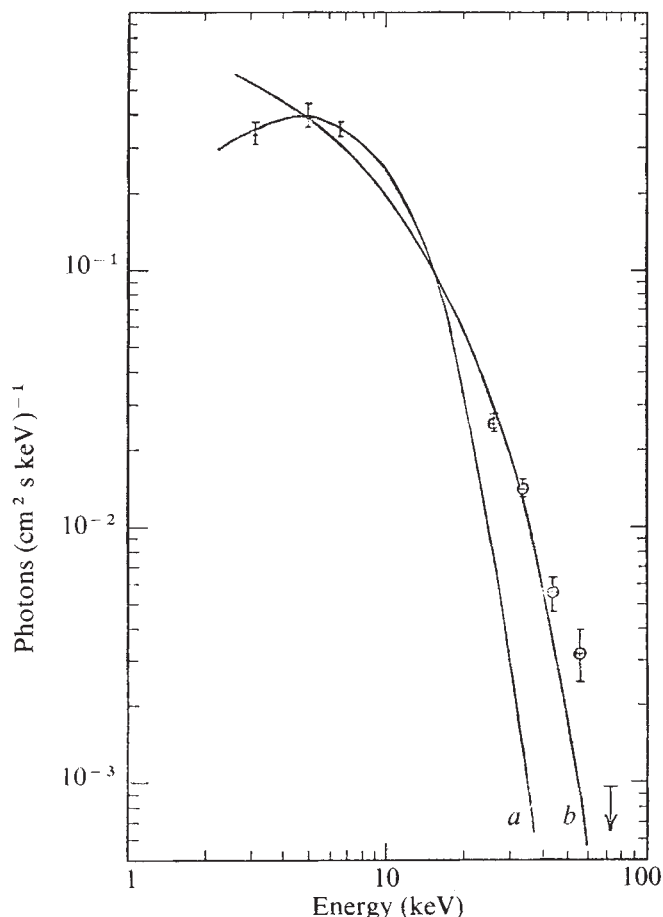
The light curve has been divided into five sections and data from each section used to obtain energy spectra. (Fig. 1a-e). Upper limits are shown at a 2σ significance level. The spectra show some evidence of steepening as the nova brightens.

Further information on the spectral development is given in Fig. 2 where results from this experiment are plotted as a ratio to the low energy measurements made with the rotation modulation collimator (RMC) experiment described in ref. 1.

Whereas the channel 1: RMC ratio (Fig. 2a) indicates some initial hardening of the spectrum the channel 4: channel 1 ratio (Fig. 2d) shows a gradual softening. The latter may also be noted in Fig. 1a-e where, after the initial section, the flux at 56 keV remains essentially constant whereas at 26 keV it increases by a factor of 3. But though the low energy intensity increases by a factor of 10 during the period covered, the results do not indicate any dramatic change in the spectrum.

In Fig. 3 we have combined the spectrum from the high energy experiment taken in period *d* (see Fig. 1) with three low energy points from the RMC experiment taken in the same period. A simple power law will not fit the data in the range shown. The results can be interpreted in terms of accretion of material from a normal star on to a compact object like a neutron star. Suppose that the X-ray flare-up is caused by a large change in the amount of accretion material available.

Fig. 3 Spectrum obtained by combining data from the high energy experiment (lower five points) with those from the RMC experiment (upper three points) measured during the same period (Fig. 1d). Errors are $\pm 1\sigma$ and 2σ for the upper limit based on counting statistics. *a*, 3 keV black body curve; *b*, 8 keV modified black body curve.



The outer layers of the accretion disk may be expected to emit low energy X rays according to a black body photon spectrum:

$$(dN/dE)_{BB} \propto E^2 / [\exp(E/kT) - 1]$$

More energetic photons produced in the deeper layers will emerge after scattering by electrons in a finite skin depth. The photon spectrum expected for this situation is ^{3,4}:

$$(dN/dE)_M \propto (dN/dE)_{BB} \{ g[1 - \exp(-E/kT)] / E^3 \}^{1/2}$$

with a Gaunt factor $g \propto E^{-0.4}$.

The deepest layers, which correspond to the greatest gravitational energy, may radiate according to a thermal bremsstrahlung law, but the overlying layers will only be transparent to the high energy tail.

Whereas a black body curve with $kT = 3 \pm 0.5$ keV fits the low energy data, a modified black body curve with $kT = 8 \pm 1$ keV is required for the high energy data. No simple single parameter curve will fit all the data. Our results therefore point to a multilayer, multitemperature source model.

Suppose that the increase in source intensity is attributable to an increasing flux, F , of material accreting on to a compact object. Then, for black body radiation, a consequence of Stefan's Law and the transfer of gravitational energy to thermal energy in a constant accretion disk geometry implies that

$$T_{BB} \propto F^{1/4}$$

and thus the spectral shape will only be weakly dependent on T_{BB} . Pringle and Rees have also found⁴ a weak dependence on F of both the modified black body temperature and the point of transition between the two laws. The relatively constant shape of the spectrum during the flare-up of the source is thus consistent with an accretion model in a binary system.

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Observations of A0535+26 with the Leicester sky survey experiment

THE Leicester University Sky Survey instrument with its field of view perpendicular to the spin axis was able to continue the study of A0535+26 after the spin axis experiments (see accompanying papers) were manoeuvred away on April 29. The source was close to the centre of our field of view on May 2, May 19 and June 1. On the first occasion the instrument was in a single channel mode (no spectral information) but eight-channel spectra between 2 and 18 keV were obtained on the second and third observations.

In addition to these three direct observations a measurable signal was obtained continuously from May 3 to May 31 while the source was outside the normal field of view of the instrument (FWHM 10°). This resulted from the brightness of the source at the higher energies; the detector efficiency combined

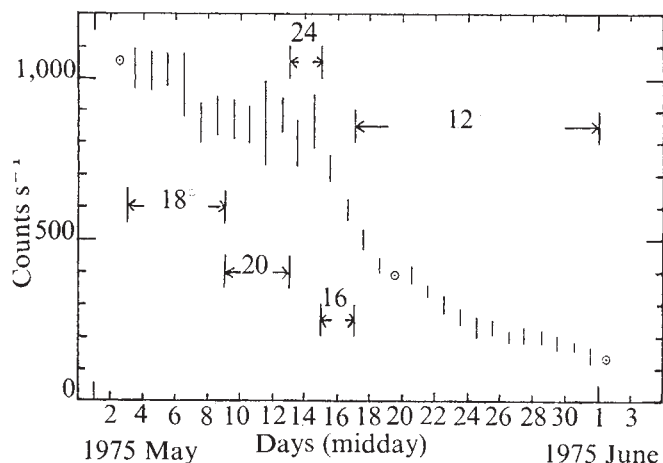


Fig. 1 The light curve of A0535+26 obtained with the Leicester University Sky Survey instrument. Points are mean daily intensities, errors are one sigma poissonian. The offsets at which the data were collected are indicated in degrees.

with the transmission through the wall of the collimator gives a mean response of $\sim 1\%$ at 12° offset decreasing to $\sim 0.3\%$ at 28° offset, for the observed spectrum of A0535+26. In this way, it has been possible to extend the Ariel V measurements of intensity to cover the whole period from the appearance of the source to June 1, leaving a gap of two days from April 29 to May 2.

The data from the various large offset observations were combined to give a continuous light curve. The method used was to correct those large angle measurements with adjacent direct measurements so that they fitted smoothly. For the data obtained between May 9 and May 17 no direct comparison was available and these data have therefore been adjusted so that the intensity changes smoothly from one offset to the next

Fig. 2 The two sets of eight-channel spectral data from A0535+26, together with predicted counts for the best fit power law spectrum. +, Observation with 1σ error; ---, best fit prediction.

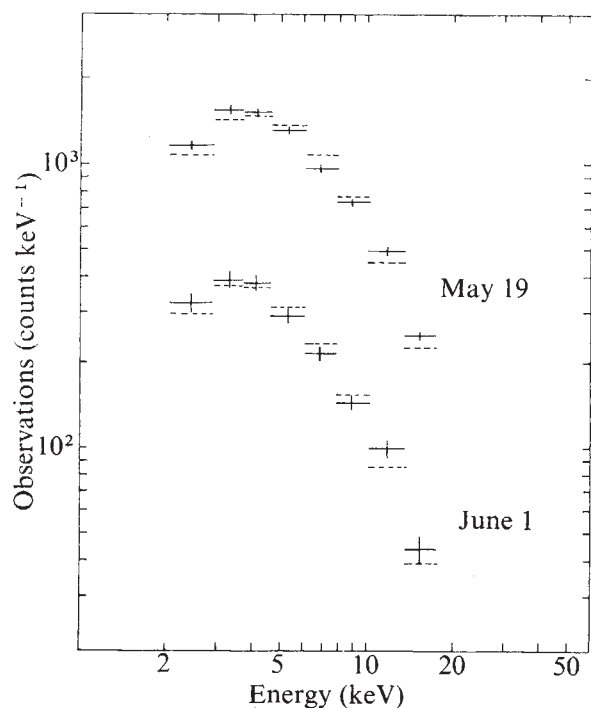


Table 1 Spectral parameters for $I = CE^{-\alpha}$ photons ($\text{cm}^2 \text{s keV}^{-1}$)

Date	Nova		Crab Nebula	
	Coefficient C	Index α	Coefficient C	Index α
May 19	1.62	-0.8	12.0	-2.1
June 1	0.67	-1.1	10.2	-1.95

Statistical error in index is 0.1.

across a manoeuvre. The resulting light curve, consisting of daily averages, is shown in Fig. 1. On May 2, we find the intensity (3–18 keV) to be three times that of the Crab Nebula. Direct comparison with the pre-maximum light curve of the MSSL/Birmingham Modulation Collimator experiment (accompanying paper) must take account of the spectral hardness of the A0535+26 source since in the energy band of that experiment (2–8 keV) the relative strength of the transient source is less pronounced.

The peak intensity of A0535+26 seems to lie between the periods covered by the two light curves, occurring between April 29 and May 2. The main features are an initial slow fall, to May 16, followed by a sharper decrease in intensity, which is apparently close to exponential, over the following 15 d.

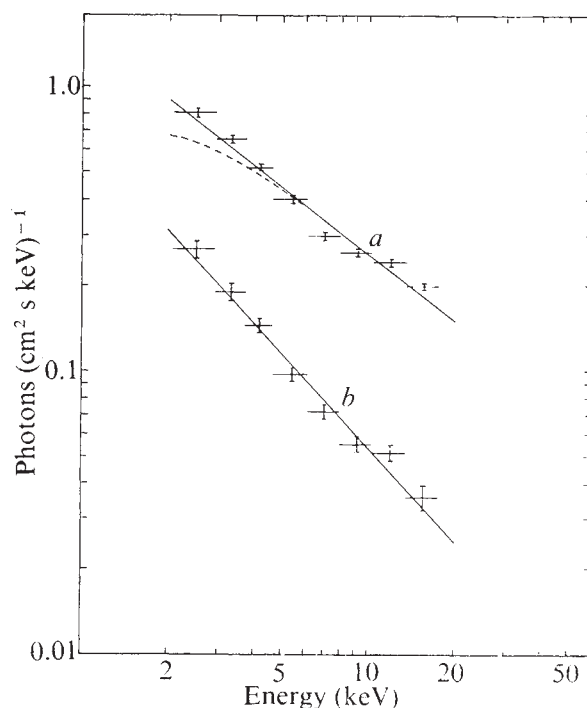


Fig. 3 The best fit input spectra with the deduced input photon spectrum. The May 19 spectrum is shown with (dashed) the effect of a low energy cutoff at 1.2 keV. a, May 19 with $E^{-0.8}$; b, June 1 with $E^{-1.1}$.

Thirty seconds of exposure to A0535+26 were obtained on May 19 in PHA mode, and 40 s on June 1. Both observations were timed to coincide with balloon observations of the source with high energy X-ray instruments (of Southampton University and MIT).

Spectral data for the two observations are plotted in Fig. 2, and Table 1 lists the optimum spectral parameters, together with those for the Crab Nebula measured simultaneously. The best fitting input spectra of A0535+26 are also shown in Fig. 3, here the relative measured intensities are given for each energy

channel, determined by comparing the observations with the input spectra folded through the detector response.

The spectrum obtained on May 19 is best characterised by a power law index of -0.8 with no absorption. The upper limit for the cutoff energy (1.2 keV) corresponds to a hydrogen column density of less than 10^{22} atom cm^{-2} . It must be emphasised that the power law fit over such a restricted energy range does not imply a non-thermal source mechanism and is used only to allow a convenient description of the spectral parameters. The June 1 spectrum is best fitted with a somewhat steeper power law of index 1.1, again with no measurable low energy attenuation. Thus if the spectrum is thermal it seems that the emission region has cooled significantly over a thirteen day period. Spectral data from the MSSL proportional counter viewing along the satellite spin axis is reported (J. Ives and J. Burnell, private communication) to show strong low energy attenuation during the rising part of the light curve, indicating that in this respect also the spectrum has changed significantly during the evolution of A0535+26.

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Decay of X-ray source A0535+26

THE All-Sky Monitor (ASM) aboard Ariel V is a pinhole camera sensitive to X rays in the energy range 3–6 keV. It has an effective area of 0.6 cm^2 with a duty cycle of $\sim 1\%$ for a given source, and monitors more than 3π of the celestial sphere each orbit¹. It is important to note that the regions of sky inaccessible to the ASM include the spacecraft spin axis and a band about the spacecraft equator, so that its field of view and those of the other five Ariel V experiments are mutually exclusive.

The transient source A0535+26 was first detected by another Ariel V experiment² during an extended spin axis hold in Taurus between days 103 and 119 of 1975, during which time a threefold increase in the emission from Cyg X-1 was observed^{3,4}. When the spin axis was moved toward Cyg X-1, the Taurus region became accessible to the ASM, enabling us to study the decay of the transient source. When Cyg X-1 was next observed by the ASM from day 136, it had returned to its usual intensity of $\sim 1/3$ the Crab Nebula in the energy band 3–6 keV.

Figure 1 is the overall 3–6 keV light curve of A0535+26 when it was in the field of view of the ASM. Before spin axis hold in Taurus, the new source was not observable at a 2σ upper limit of $\sim 10\%$ of the Crab Nebula intensity (the latter was simultaneously measured at its stationary value of $1.4 \text{ cm}^{-2} \text{ s}^{-1}$). When first observable by the ASM on day 119, (April 29) the new source was $\sim 15\%$ more intense than the Crab Nebula, and stayed at this level for approximately one week. The source then decayed with an e -folding time of $\sim 19 \text{ d}$ until the Sun was too close to the source for unambiguous separation. When the Sun moved sufficiently far to allow the intensity of the source to again be interrogated, it was found to be unobservable (at $< 10\%$ of the Crab Nebula).

The plateau region of the decay measured before day 126 is not unusual for transient X-ray sources. Similar plateau

effects in the decay just after maximum have been observed in the older transient sources Cen X-4 (ref. 5) and 2U1543–47 (ref. 6), as well as in the Ariel V transient source A1524–62 (ref. 7). Unlike these sources, however, the e -folding time for the Taurus source is considerably shorter than the $\gtrsim 2$ months which is typical of the others. The only other transient sources previously reported with comparably short lifetimes are 3U1735–28 and A1118–61, with which the Taurus source shares spectral and temporal (pulsing) similarities (see accompanying papers and ref. 8). No temporal resolution finer than 100 min is possible with the ASM, nor is there any spectral resolution obtainable in the 3–6 keV acceptance window. The window is, however, insensitive to input spectral form¹, so that the light curve displayed for this band in Fig. 1 does not suffer from any spectrum-dependent systematic offset.

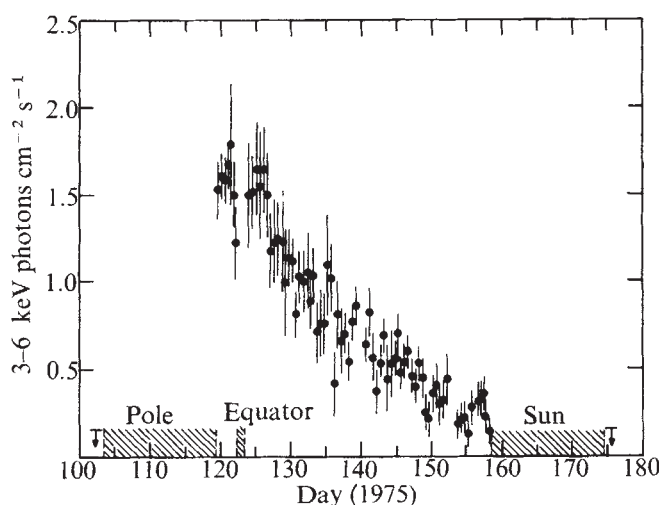


Fig. 1 Intensity measured from A0535+26 over approximately half-day accumulation times. The source is not in the usable field of view of the ASM where the abscissa is shaded. The 2σ upper limits before and after the measured points are determined primarily by the statistical accuracy in the measurement of the Crab Nebula, with which A0535+26 may be confused in the ASM data.

Finally, we note that the short-lived transients typified by 3U1735–28, A1118–61 and A0535+26 may be as much as an order of magnitude more frequent than longer-lived transients. The proximity to the galactic plane argues that these sources have a Population I distribution, and are $> 1 \text{ kpc}$ distant. As discussed in ref. 7, their short lifetimes (and appearances in relatively congested regions of the plane) make them less easily detectable than the longer-lived transients. With an average luminosity at maximum of $\sim 10^{37} \text{ erg s}^{-1}$, their frequency may approach 100 yr^{-1} in the Galaxy without conflicting with existing source or galactic ridge measurements. L. J. K. acknowledges support from the University of Maryland.

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Dynamo generation in Mercury

OBSERVATIONS during the most recent encounter of the planet Mercury by Mariner 10 seem to have confirmed the existence of a small but intrinsic magnetic dipole of about 3×10^{-3} gauss R_M^3 , where R_M is the radius of Mercury¹. This field may be small enough to have a non-dynamo explanation^{1,2}, but it is nevertheless of interest to examine whether dynamo generation is possible for the present Mercury.

Siegfried and Solomon³ have calculated models for the interior and thermal evolution of Mercury. In these models, the cosmochemical calculations of Lewis⁴ were used to constrain the composition. They concluded that Mercury is most likely differentiated⁵, with a Fe-Ni core extending out to about $0.7R_M$, and a silicate mantle. I have tested their models for four necessary (but possibly not sufficient) conditions for magnetohydrodynamic dynamo generation.

First, dynamo generation requires that the interior be at least partially fluid, since solid-state convection is too slow for dynamo generation. In the models of Siegfried and Solomon, radioactive heat sources in the silicate mantle may be sufficient for fluidity in a region extending from about 200 km to about 800 km below the planet surface. The metallic core is either partly or entirely solid, depending on when differentiation took place.

Second, dynamo generation requires an energy source that drives a flow of the fluid relative to a rigidly rotating planet. Thermal convection seems to be the only plausible energy source, since precession is insignificant², and the other possible sources are just as unsatisfactory for Mercury as they are for the Earth⁶. The Siegfried and Solomon models assume that all the radioactive heat sources are in the mantle, so that thermal convection occurs in a 600 km thick silicate layer, but not in the stably stratified metallic core. Other heat sources in the core (latent heat, gravitation) seem to be insufficient to drive the temperature gradient superadiabatic and initiate convection. The thermal conductivity of the metallic core is sufficiently high that convection would only occur if about half of the radioactive heat sources reside in the core. One possibility is that Mercury has a similar fraction of ⁴⁰K as Lewis⁴ proposes for the Earth's core. This would violate Lewis's cosmochemical arguments, since potassium compounds are too volatile to condense from the primitive solar nebula at Mercury's distance from the Sun.

Third, dynamo generation requires that the magnetic field diffusion time, τ_M , exceeds the characteristic fluid flow (convective) time scale τ_C . The ratio of τ_M to τ_C is known as the magnetic Reynold's number. In the convecting silicate layer, simple mixing length theory⁷ predicts a convective velocity of 0.1 cm s^{-1} for the expected heat flux³ of about $50 \text{ erg cm}^{-2} \text{ s}^{-1}$. A similar estimate applies to the Earth's outer core⁸ and is unlikely to be incorrect by more than an order of magnitude. The corresponding convective time scale is $\tau_C \sim 1\text{--}10 \text{ yr}$. The magnetic field diffusion time is given by²

$$\tau_M \approx 10^{-9} \sigma l^2 \text{ s} \quad (1)$$

where σ is the electrical conductivity in $\Omega^{-1} \text{ cm}^{-1}$ and l is the smallest dimension of the convection region. The requirement $\tau_M > \tau_C$ implies $\sigma > 10\text{--}100 \Omega^{-1} \text{ cm}^{-1}$. This would be easily satisfied in the core ($\sigma \sim 10^3$ or $10^4 \Omega^{-1} \text{ cm}^{-1}$) but not in the microconducting silicates. In the Siegfried and Solomon models,

the temperature is at most about 2,100 K in the mantle. At this temperature, the conductivity in the Earth is probably less than $0.1 \Omega^{-1} \text{ cm}^{-1}$ (ref. 9). Cosmochemical arguments⁴ indicate that the Mercurian mantle should be somewhat different from the Earth, with MgSiO_3 prominent, but a generous extrapolation of high temperature laboratory experiments on enstatite¹⁰, indicates that a conductivity in excess of $1 \Omega^{-1} \text{ cm}^{-1}$ is very unlikely. This figure is for solid materials, but these minerals (unlike pure germanium or silicon) are not likely to change their short range order or conductivity dramatically upon melting¹¹. (The conductivity may increase by as much as an order of magnitude, but certainly not several orders of magnitude.) I conclude that the liquid silicate conductivity is probably inadequate for dynamo generation. I note that generation in a thin silicate layer near the surface of the planet is likely to lead to a substantially non-dipolar field at the planetary surface.

Fourth, dynamo generation requires a fluid flow of sufficient complexity to satisfy Cowling's theorem¹². In particular, the requirement of a non-axisymmetric field is usually interpreted to imply a need for "rapid rotation" of the planet (although other alternatives may exist¹³). Actually, "rapid rotation" only means that the Coriolis force has an important effect on convective flow. A measure of this is the ratio of inertial to Coriolis forces, $v/\Omega l$, where $v \sim 0.1 \text{ cm s}^{-1}$ is a typical convective velocity in the absence of rotation, and $\Omega \sim 1 \times 10^{-6} \text{ s}^{-1}$ is the planetary angular velocity. For $l \lesssim 10^5 \text{ cm}$, this ratio (which is a nominal Rossby number for the flow) is less than unity, and the Coriolis force is important. For the largest scale convective flows in Mercury, $l \approx 10^7 \text{ cm}$, so that in this respect Mercury is a rapidly rotating planet. The requirements of Cowling's theorem may then be satisfied because of the effect of planetary rotation alone.

In a more complete theory, such as that attempted by Parker¹⁴, and more recently in rigorous work by Busse (unpublished), the criterion for dynamo generation is generally more stringent than the simple ones we have discussed. A complete theory would also make a prediction for the field magnitude. A feature of the Busse theory is that if the core is convecting, then the small field of Mercury would be a consequence of the slow planetary rotation. Unlike the Earth, the ohmic dissipation in Mercury is likely to be completely insignificant compared with any important energy source such as radioactivity.

I conclude that a literal interpretation of the cosmochemical calculations of Lewis⁴ implies that dynamo generation in Mercury is improbable. Generation would be possible if the metallic core were contaminated with substantial amounts of radioactive material. It would then follow that not all the material that comprises Mercury condensed from the solar nebula at the present distance of Mercury from the Sun. The magnitude of the field and any future multipolar analysis (such as might be achieved by an orbiter) will be important in determining the field source.

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Fracture mechanism of Henbury meteorite by separation along surfaces of shear faulting

THE smallest Henbury meteorite crater contained¹ a massive iron that had broken into four pieces weighing 132.7, 54.4, 10.9 and 2.3 kg, respectively. When these four pieces were excavated from the depths of the crater they were found to be still juxtaposed to one another. We have studied a 5 kg, 20×40 cm slice (BM 1932, 1359) taken from the largest of these pieces. We have also prepared and studied sections cut from a number of pieces of 'shrapnel' from a Henbury crater, weighing about 100 g and purchased from the American Meteorite Laboratory.

The macrosection BM 1932, 1359 is traversed by a number of fault displacements, most of which are continuous across the whole section and lie approximately parallel to an outer edge of the section. This suggests that, in this instance, the fracture surfaces could represent the extreme situation in which limited shear displacement gives way to physical separation along the fault surface. This macrosection is too large for satisfactory microscopic examination, and, consequently, the fracture mechanism was explored using the smaller shrapnel fragments. In each instance slip-fault displacements were encountered within the shrapnel pieces, and in one instance an internal crack was found to merge continuously into a pronounced displacement fault. The most convincing evidence for fracture by separation along the surfaces of shear faults, however, comes from those pieces of shrapnel that were not reheated significantly during break-up. In those cases, the bulk of the kamacite (α Fe-Ni) is shock hardened but not recrystallised. Shear-displacement surfaces within these specimens were revealed (by Nital etch) as continuous traces of fine grained (1–5 μ m) recrystallisation in the kamacite, similar to geological mylonitisation zones. The 'throw' of the fault varies from a few micrometres to several millimetres and is best seen where particles of taenite (γ Fe-Ni) are cut and displaced. The Nital etch did not reveal recrystallisation in the taenite and, consequently, the metallographically visible 'thickness' of the shear effect in taenite was limited to the apparently sharp geometrical surface of displacement. But the heat that is generated during the rapid propagation of the displacement fault causes the shock-hardened kamacite to recrystallise within a zone of finite thickness on either side of the surface of displacement. The visible width of this recrystallised zone varies from 1 to 20 μ m according to the energy input at the particular fault.

We have examined a number of sections of Henbury 'shrapnel' in which relics of this 'mylonitised' kamacite are present at the outer fracture surfaces. To identify these relics of heat effect with shear mylonitisation, it is necessary to eliminate the possibility that the surface heating was produced by ablative friction during high-velocity flight through the atmosphere². It is possible to do this since an ablative heat alteration zone³ is composed of coarse ragged α_2 (martensitic) product that indicates reheating above 850 °C, whereas, by contrast, the effect observed at the surface of Henbury shrapnel is a fine-grained, recrystallised α (non-martensitic): the product of a much milder heating of shock-hardened kamacite.

The presence of shocked kamacite in Henbury shrapnel indicates that the fragmentation took place under conditions of shock loading with a shock pressure of at least 130 kbar. The shear faults seem to be superimposed upon the shock-hardened structure and, therefore, seem to have formed subsequently.

Once the fault has commenced to move on a selected surface it is likely to continue to do so because of the local generation of heat and the superplastic lubrication of the small kamacite grains in the mylonitised zone. The factors that cause a

particular surface to be selected for shear faulting are, however, more difficult to specify, but, in general terms, they must arise from unbalanced shear forces as the projectile adjusts itself rapidly to the complex array of shock waves that accompany high-velocity impact with the Earth.

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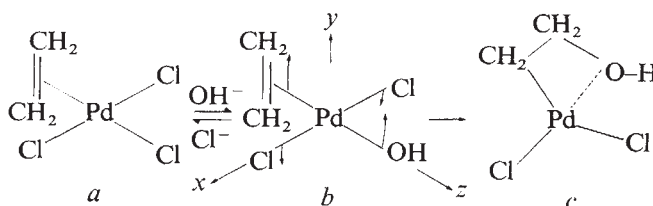
Mechanism of the oxidation of ethylene by palladium(II)

THE oxidation of ethylene to acetaldehyde by aqueous palladium chloride forms the basis of the Wacker process. The kinetics and mechanism of this reaction have been extensively studied for ethylene and other olefins, and most authors assume that the formation of a *cis* hydroxoethylene-palladium complex is an essential step in the reaction^{1–4}. We propose here a new mechanism by which the *trans* intermediate, $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$, may directly yield the palladium σ -hydroxyalkyl species without the necessity of *trans*-*cis* isomerisation or the attachment of a fifth ligand.

It is widely accepted that the first steps of the reaction are the formation of a π -ethylene complex, $[\text{C}_2\text{H}_4\text{PdCl}_2]^-$ (Fig. 1a) the palladium analogue of Zeise's salt, followed by the aquation and dissociation of the *trans* aquo complex to give *trans* $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$ (Fig. 1b). A *trans* to *cis* isomerisation is then assumed with the subsequent insertion of the ethylene into the palladium-hydroxide bond (a *cis*-migration rearrangement) giving a palladium σ -hydroxyalkyl molecule $[\text{PdCl}_2\text{CH}_2\text{CH}_2\text{OH}]^-$ (Fig. 1c) which yields acetaldehyde by a palladium assisted hydride shift. Little attention^{5,6} has been focused on the isomerisation of $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$ although it has been suggested^{1,7} that a dihydroxo species is involved as an intermediate. An alternative view^{4,8,9} is that a five-coordinate palladium(II) species is involved, thus avoiding the requirement of the *cis* configuration. The kinetic evidence for these latter proposals has been obtained at low chloride and acid concentrations and is somewhat unconvincing in the normal concentration range so that the isomerisation of $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$ remains controversial.

We have already suggested¹⁰ a model for catalysis by nickel (II) complexes, which was based upon the facilitation of certain molecular vibrations by the vibronic coupling of the highest filled molecular orbital with vacant molecular orbitals derived

Fig. 1 Compounds occurring during the oxidation of ethylene by palladium(II): a, π -ethylene complex; b, *trans* $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$; c, the palladium σ -hydroxyalkyl molecule $[\text{PdCl}_2\text{CH}_2\text{CH}_2\text{OH}]^-$.



from ligand π -acceptor orbitals. Application of that model to the Wacker process is straightforward. The *trans* complex $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$ is a planar molecule with C_{2v} molecular symmetry. The out-of-plane YMZ bending vibration of a MX_2YZ molecule of C_{2v} symmetry with the coordinate axes as shown in Fig. 1 has b_2 symmetry. If this vibration is facilitated in $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$ then the C_2H_4 and OH^- ligands will move closer together as the molecule distorts towards a pseudotetrahedral structure allowing a ready path for the insertion of ethylene into the Pd–OH bond. Molecular orbital calculations are available^{11,12} for Zeise's salt, $[\text{C}_2\text{H}_4\text{PtCl}_3]^-$, and because of the marked similarity of the electronic structures of PtCl_4^{2-} and PdCl_4^{2-} (ref. 13) we assume that the general qualitative features of the Zeise's salt calculations will be applicable to $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})\text{Cl}_2]^-$. Both calculations agree that the highest filled molecular orbital is of b_1 symmetry, although the exact nature of the orbital is debatable. Facilitation of the b_2 vibration may occur by the vibronic coupling mechanism if a vacant molecular orbital of a_2 symmetry ($b_1 \times b_2$) lies close enough to the filled b_1 orbital to lead at least to a reduction in the force constant of the b_1 vibration. Such an orbital is available: it is largely a non-bonding orbital of the metal complex derived from the molecular orbitals of the ethylene ligand. Symmetry considerations are thus consistent with the possible facilitation of the b_2 molecular vibration and the ethylene and hydroxide ligands may approach each other by the out-of-plane bending of the $[\text{C}_2\text{H}_4\text{Pd}(\text{OH})]$ unit. Attack by the hydroxide on the π^* orbitals of ethylene may occur either in an approximately end-on situation with the carbon atoms and the oxygen atom lying in the *zy* plane or, if the ethylene molecule is allowed to rotate about the palladium–ethylene axis, at the centre of the double bond. If, as suggested by Hartley¹⁴, the palladium–olefin bonding is weaker than the platinum–olefin bond then attack on the largely vacant π^* orbitals may be more likely leading to *cis*-insertion through a four membered metalocycle.

This mechanism provides an alternative to the *trans*–*cis* isomerisation which, together with rotation of the olefin, is a prerequisite for the *cis*-insertion reaction which is generally accepted at present.

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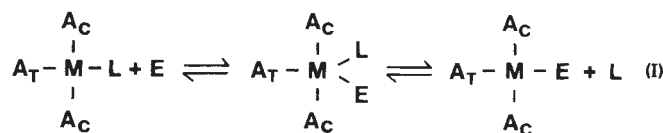
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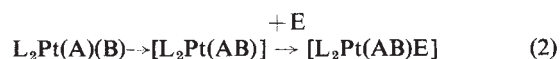
complexes should have either 16 or 18 electrons in the transition metal's valence shell. We point out here that though substitution reactions at platinum(II) occur by an associative route, reactions between ligands coordinated to platinum(II)—here called combination reactions—occur by a dissociative route that violates the '16 and 18 electron rules'.

Substitution reactions involve a trigonal bipyramidal transition state (reaction 1) and not a square pyramidal one, and in the trigonal bipyramidal transition state the leaving group



(L), the entering group (E) and the *trans*-ligand (A_T) all lie in the trigonal plane with angles of about 120° separating them.

Combination reactions, such as that involved in the insertion of olefins into platinum(II) hydride bonds to give platinum(II)-alkyls^{4,5} and that involved in the insertion of carbon monoxide into platinum(II)-alkyl bonds to give acyl complexes^{6,7}, occur by a dissociative route (reaction 2) in which insertion occurs



first to give an intermediate that is either 3-coordinate or 4-coordinate with the fourth coordination site occupied by a solvent molecule.

A similar mechanism has been suggested for the insertion of isocyanides into platinum(II)-alkyl and aryl bonds⁸, and

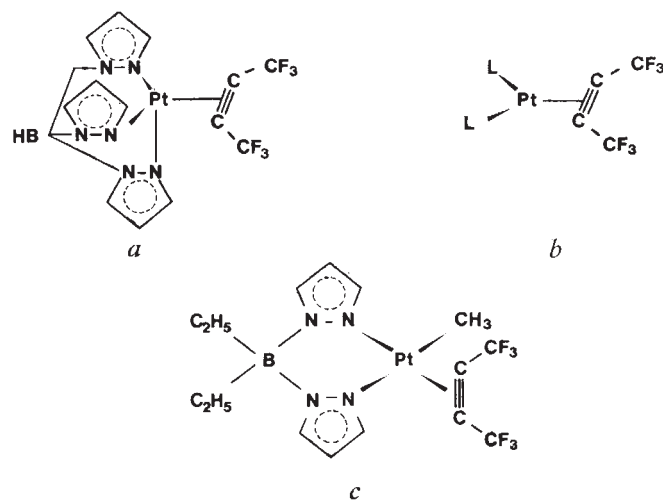


Fig. 1 Platinum(II) (methyl) acetylene complexes (see text).

it is likely that the insertion of olefins and acetylenes into platinum(II)-alkyl bonds follows the same route since an essential prerequisite for reaction is a 4-coordinate complex in which the alkyl and olefin groups are *cis* with respect to each other⁹. It is noteworthy in this connection that 5-coordinate complexes in which the alkyl and acetylene groups are *cis* with respect to each other are reluctant to undergo insertion. Thus the five-coordinate complexes in Fig. 1a, in which the Me–Pt–acetylene bond angle equals 88.5° (ref. 10), and in Fig. 1b, where $\text{L} = \text{PMe}_2\text{Ph}$, do not give insertion, whereas the five-coordinate complex in which $\text{L} = \text{AsMe}_2\text{Ph}$ (Fig. 1b), and the four-coordinate complex shown in Fig. 1c, do give insertion.

Nucleophilic attack on olefins coordinated to platinum(II) and palladium(II) can occur either on the same side as the

Mechanisms of reactions at square planar metal centres

SQUARE-PLANAR platinum(II) complexes have in the past been found to react by associative mechanisms (see refs 1 and 2). This has been of some comfort to the proponents³ of the '16 and 18 electron rules', which state that all stable transition metal complexes and intermediates in the reactions of such

metal (*cis*-attack; Fig. 2a) or on the side remote from the metal (*trans*-attack; Fig. 2b). *Cis*-attack will almost invariably involve prior coordination of the nucleophile to the metal. This coordination can, in principle, occur in two ways: first, by displacement of one of the ligands bound in the square-plane *cis* to the olefin giving a 4-coordinate intermediate; second, at one of the two axial sites giving a 5-coordinate intermediate. Analysis of the available evidence would suggest that *cis*-attack always involves the first of these two possible mechanisms. Thus *cis*-attack has been shown by stereochemical analysis of the products to occur when phenyl^{12,13}, carboxymethyl¹², chloride (sometimes)^{14,15} and acetate (in the absence of chloride)¹⁶ are the attacking nucleophiles. Kinetic investigations of the chloride¹⁴ and acetate¹⁶ reactions have shown that *cis*-attack involves reaction of a ligand originally bound to a site in the square-plane and does not involve an extra ligand coordinated to an axial site. Thus, *cis*-nucleophilic attack on olefins involves a mechanism essentially similar to that given in reaction 2 for the insertion type of combination reaction.

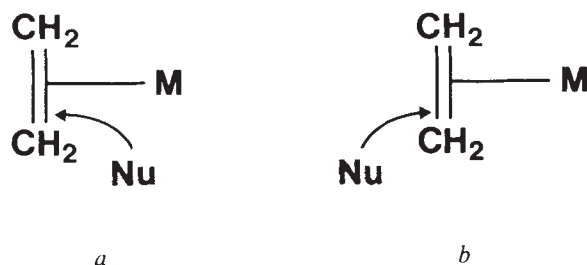


Fig. 2 Possible modes of nucleophilic attack on metal-olefin complexes: a, *cis*-attack; b, *trans*-attack.

Clearly, it is of interest to ask why substitution reactions involve a trigonal bipyramidal intermediate whereas combination reactions apparently involve reaction between two ligands bound in the square-plane. Though no definitive answer can be given two salient points can be made:

First, ligands bound in the square-plane lie closer to one another ($\sim 90^\circ$ apart) than ligands in a trigonal bipyramidal intermediate where the incoming ligand and the ligand being attacked would presumably lie in the trigonal plane ($\sim 120^\circ$ apart) by analogy with their geometry in substitution reactions. The fact that the methyl and acetylenic ligands in the trigonal bipyramidal complex (Fig. 1a) do not combine is of interest in this connection as they lie at almost 90° to each other. It is, however, possible that in this case reaction is not favoured electronically.

Second, coordination of ligands in the square-plane clearly involves a much stronger interaction between the ligand and the metal than does axial coordination. This facilitates combination reactions since coordination weakens the multiple bonds in unsaturated ligands such as carbon monoxide, olefins and acetylenes, because of the π -back donation of electron density to the ligand antibonding orbitals. That this bond-weakening is important is demonstrated by the stability of $[(Et_3B(pz)_2)Pt(Me)(ac)]$ (Fig. 1c), where ac is $PhC\equiv CMe$ or $PhC\equiv CPh$, compared with the rapid insertion that occurs when attempts are made to prepare the analogous complex with the strongly π -accepting acetylene, $CF_3C\equiv CCF_3$ (ref. 9).

It is far from clear why both substitution and combination reactions, on the basis of current experimental evidence, seem to spurn the square-pyramidal intermediate, particularly in view of the low energy barrier separating it from the trigonal bipyramidal configuration¹⁷.

Nucleophilic attack on olefins coordinated to metal ions

occurs most readily when palladium(II) is the activating metal ion; other metal ions such as platinum(II) are much less active as promoters. A number of reasons have been advanced¹⁸ to account for this.

First, palladium(II)-olefin complexes may be formed more rapidly than platinum(II)-olefin complexes.

Second, π -back donation from metal to olefin is likely to be less in the palladium(II)-olefin complex than in the platinum(II)-olefin complexes because of the higher ionisation potentials of the valence shell electrons of palladium(II). Thus, the electron density around the olefinic double-bond would be less when it was coordinated to palladium(II) than to platinum(II), and so olefins coordinated to palladium(II) would be more susceptible to nucleophilic attack.

Third, the palladium(II)-olefin bond is weaker than the platinum(II)-olefin bond so that the activation energy of any rearrangement process that may be necessary during the course of the reaction will be lower for palladium(II)-olefin complexes than for platinum(II)-olefin complexes.

Fourth, palladium(II) can more readily expand its coordination sphere to accept a fifth and sixth ligand than can platinum(II). This would allow the incoming nucleophile to coordinate to palladium(II) before attacking the olefin, thus lowering the activation energy of the nucleophilic attack.

The fact that palladium(II)-olefin complexes such as $[(C_2H_4)PdCl_2]_2$ (ref. 19) and $[Pd(CH_2=CH-CH_2NH_3)Cl_3]$ (ref. 20) are hydrolysed instantaneously, whereas their platinum(II) analogues^{21,22} are hydrolysed, respectively, only on heating and not at all, suggests that though the first of those reasons may be important, it is by no means the limiting factor. Kinetic and stereochemical evidence suggests that the last reason is irrelevant because coordination of the attacking nucleophile to a fifth position out of the square-plane is abortive and does not precede the combination reaction between olefin and nucleophile.

We have recently carried out experiments to distinguish between the remaining reasons using methoxide as the nucleophile, as it is known to attack olefin complexes *trans* with respect to the metal²³, thus eliminating any complications caused by the greater preference of this nucleophile for coordination to one or other metal.

1. When platinum(II)-olefin complexes such as $[(PPhEt_2)PtCl_2(C_2H_4)]$ are treated with methoxide ion, the principle reactions involve displacement of ethylene from the platinum(II) complex to yield either $[(PPhEt_2)PtCl_2]_2$, or $[(PPhEt_2)PtCl_2(OMe)]$, which subsequently decomposes with the elimination of formaldehyde; nucleophilic attack on the olefin to yield methylvinylether is only a minor reaction²⁴.

2. Treatment of $[(C_2H_4)MCl_2]_2$ with methanolic sodium carbonate gave a 70% yield of $CH_3CH(OMe)_2$ and no detectable C_2H_4 , $CH_2=CHOMe$ or $HCHO$ when M was palladium; but when M was platinum only C_2H_4 and $HCHO$ (formed by decomposition of the initially formed platinum(II)-methoxy complex) were detectable. $[CH_3CH(OMe)_2]$ is formed by nucleophilic attack by MeO^- at the β -carbon atom of $-Pd-CH_2-CH_2OMe$ formed by nucleophilic attack on the coordinated ethylene. Both experiments indicate that platinum(II)-olefin complexes readily undergo substitution reactions with methoxide ion, which suggests that the third reason outlined cannot be of major importance in determining the relatively low susceptibility of platinum(II)-olefin complexes to nucleophilic attack. It thus seems that palladium(II)-olefin complexes are highly susceptible to nucleophilic attack because the correct balance is achieved between the loss of electron density from the olefin π -bonding orbital through the olefin to the metal σ -bond, and the gain of electron density in the olefin π^* -antibonding orbital through the metal to the olefin π -bond. Too much π -back donation prevents nucleophilic attack, as shown by platinum(II) and even more effectively by platinum(0), and too little π -back donation is equally effective in preventing nucleophilic attack as shown by silver(I).

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Table 2 Coefficients of isotopic ions in binary diffusion at 25 °C

Diffusing isotope	Medium	Mean $D \times 10^9$ ($\text{m}^2 \text{s}^{-1}$)	No. of expts	Average deviation from mean
$^{134}\text{Cs}^+$	0.5 KCl diffusing into water	1.871	3	± 0.002
$^{137}\text{Cs}^+$		1.872	3	± 0.003
$^{125}\text{I}^-$	2.0 M KI diffusing into water	2.062	3	± 0.003
$^{131}\text{I}^-$		2.064	4	± 0.002

solvents, and in view of the reported separation factors the difference in diffusion coefficients may be expected to be measurable. I report here the results of experiments designed to test this supposition.

The heavy ions chosen for study were the pairs $^{125}\text{I}^-$, $^{131}\text{I}^-$ and $^{134}\text{Cs}^+$, $^{137}\text{Cs}^+$; in the former case the absolute mass difference is double that in the uranyl ion case. Both these ionic species are considered to be unhydrated so that their effective masses in aqueous solution are known fairly reliably. Diffusion coefficients for these ions were measured using the magnetically stirred diaphragm cell method⁵. The conditions which were varied in the course of these experiments were temperature, concentration and the type of diffusion. One set of tracer-diffusion results is shown in Table 1. In this first type of experiment, the solutions were uniform throughout the cell and there was a gradient in the trace radioactive components only.

Two further sets of binary diffusion experiments were also carried out. In these experiments there was a gradient in the salt concentration as well as in the radioactive component. In one case, 0.5 M KCl was allowed to diffuse into water and radioactive Cs^+ ions were added as tracer; in the other, 2.0 M KI diffused into water accompanied by radioactive I^- ions. It will be appreciated that the conditions of these two sets of experiments approach more closely those used by Miller⁴ than do the tracer-diffusion set used in the earlier experiments. The results are shown in Table 2.

The results (Tables 1 and 2) indicate that within the 'average error' of about 0.2% there is no detectable isotope effect for these heavy ions under a variety of conditions. Each experiment in which both KCl and trace Cs^+ ions were diffusing into water could be internally calibrated since the diffusion coefficients of the system KCl-water were accurately known⁵. This refinement allows diffusion coefficients to be specified to within $\pm 0.1\%$ for this system.

The high separation factors reported in Miller's original paper⁴, particularly those recorded when agar gel was used as the diffusion medium, were caused by transient diffusion effects and were not related directly to the steady state diffusion coefficients (L. Miller, personal communication). Miller⁶ has

Search for isotope effects in heavy-ion diffusion in liquids

THERE is some controversy as to the existence of isotope effects in liquid diffusion in which isotopically labelled molecules diffuse in the same liquid medium. Friedman¹ has predicted from the standpoint of the correlation-function theory of diffusion that there should be little or no effect and certainly nothing approaching the inverse square mass relationship and this has been shown^{2,3} to be true for organic liquids. Miller⁴ claims, however, that uranyl ions diffusing in aqueous solution do show an isotope effect and also that heavier ions diffuse faster than lighter ions. He attributed the phenomenon to kinetic effects in the molecular diffusion process; in particular, to persistence of velocities and backscattering. The effects should apply generally to heavy ions diffusing in light

Table 1 Tracer-diffusion coefficients of isotopic heavy ions in aqueous solution

Diffusing isotope	Medium	Temperature (°C)	Concentration (mol l^{-1})	Mean $D^* \times 10^9$ ($\text{m}^2 \text{s}^{-1}$)	No. of experiments	Average deviation from mean
$^{125}\text{I}^-$	KI	10	0.5	1.381	3	+0.004
$^{131}\text{I}^-$	KI	10	0.5	1.377	3	± 0.006
$^{125}\text{I}^-$	KI	10	3.0	1.393	5	+0.002
$^{131}\text{I}^-$	KI	10	3.0	1.394	5	± 0.002
$^{125}\text{I}^-$	KI	25	0.5	1.992	4	± 0.005
$^{131}\text{I}^-$	KI	25	0.5	1.985	5	± 0.007
$^{125}\text{I}^-$	KI	25	2.0	1.952	6	± 0.004
$^{131}\text{I}^-$	KI	25	2.0	1.950	5	± 0.004
$^{125}\text{I}^-$	KI	45	0.5	2.899	4	± 0.010
$^{131}\text{I}^-$	KI	45	0.5	2.900	3	± 0.003
^{134}Cs	KCl	25	0.5	1.935	5	± 0.003
^{137}Cs	KCl	25	0.5	1.930	4	± 0.002

derived from the transient separation data the difference between the diffusion mobilities of $^{235}\text{UO}_2^{2+}$ and $^{238}\text{UO}_2^{2+}$ ions and found it to be of the order of 0.25%. This latter result is compatible with those reported here because were a persistence mechanism responsible, it would affect less those ions which have a mass considerably below that of the uranyl ion. In any event, both studies show that isotopic effects in the diffusion of heavy ions in light solvents are either nonexistent or very small.

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Stereoillusion based on visual persistence

If a moving vertical line is stroboscopically lit, it seems to undergo a multiplication, so that at any instant of time it is perceived as a series of lines, spread out in the direction of motion¹⁻³. If the illumination going to the eye is reduced, the apparent multiplication increases³, suggesting possibly that the effect is a function of sensory integration time or "persistence" as it is affected by adaptation state of the eye². Since the adaptation conditions of the two eyes are substantially independent⁴, it is interesting to ask what we should see if a stroboscopically moving target were viewed with a filter over one eye only, and the different numbers of apparent bars stereoscopically fused. In these conditions more bars should be signalled by the filtered than by the unfiltered eye, and it is not obvious what the fused percept will be.

To investigate this problem I used a haploscopic display in which the number of bars presented separately to the two eyes was controlled on an oscilloscope (CRO) display by a digital computer. One eye was presented with a single vertical bar (5° high) that moved in discrete steps of 0.67° every 40 ms. The bar can be thought of as a target moving at 1.0 Hz, peak-to-peak amplitude 9.5°, and stroboscopically illuminated at 25 Hz. The other eye was presented with two bars, one of which corresponded in spatial position with the bar in the first eye, and the second with the preceding position of that bar (Fig. 1). Fusion of the left and right eye displays was aided by means of a stereoscope positioned in front of the CRO. Observers were instructed not to "track" the apparently moving targets, and to aid steady fixation a vertical white (0.4 log foot lambert) line was provided in the optical plane of the CRO screen.

The appearance of the fused display was that of several bars travelling in an orbit around the fixation line. The number of lines was difficult to count accurately, but it was certainly often in excess of two. These bars moved as a unit, giving an impression of a picket fence in motion. There was a convincing impression of depth to the motion, the "fence" appearing to rotate in a clockwise orbit (as if viewed from above) with the extra bar in the left eye, and anticlockwise with the extra bar in the right eye. To demonstrate this, a random series of trials was programmed in which the extra bar appeared in the left and right eye, and observers (colleagues and undergraduates in the laboratory) pressed a button on each trial to indicate whether they saw clockwise or anticlockwise motion. The experimenter was not in the room. Responses of the observers very seldom varied from the rule that an extra left-eye bar produced clockwise motion, and an extra right-eye bar anticlockwise motion.

The direction of this effect is the same as that of the "Pulfrich

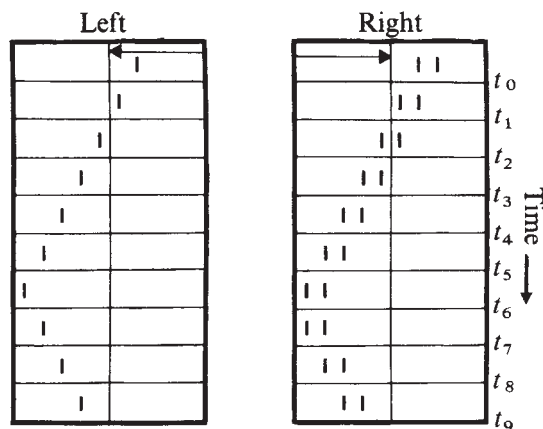
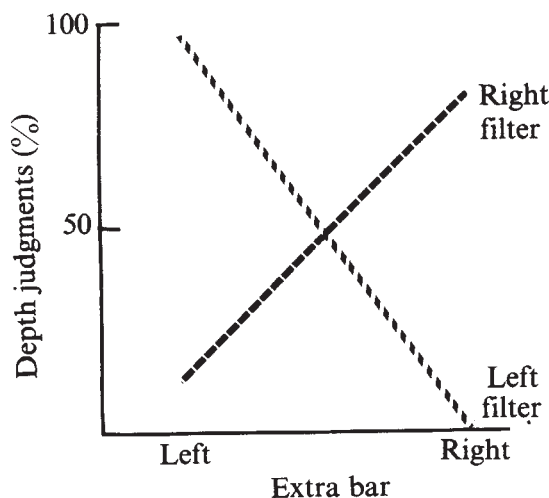


Fig. 1 Schematic representation of the display at successive times ($t_0, t_1 \dots t_9$). In the actual display the left and right halves were presented separately to the left and right eyes and fused to give a single percept. In this particular representation the right eye has an extra bar, which is in a "trailing" position.

effect"⁵, in which a continuously visible target seems to move in a clockwise orbit when viewed with a left-eye filter. If we recall that a reduction in illumination produces greater visual persistence, the facts are strongly suggestive of a common mechanism for the Pulfrich stereophenomenon and the new illusion reported here. To support this hypothesis, a neutral density alter in front of one eye was added to the display described above. With a suitably chosen filter (1.0 log units) in front of the left eye we expect depth to be enhanced when the extra bar is in the left eye, and reduced when it is in the right eye; conversely with the filter over the right eye. Observers were given a random mixture of trials with extra bar in the left and right eyes, first with the filter in front of one eye (40 trials) and then with the other eye filtered (40 trials). On each trial they were asked to say whether they saw clear depth (and in which direction) or whether the effect was weak. Since all observers had previously seen the strong effect without a filter, and since it was very quickly obvious to them that depth was absent or much reduced on a proportion of the trials, these judgments were accomplished without difficulty. The results, shown in Fig. 2, were as expected; clear depth was seen only when the Pulfrich, (filter) and persistence (extra bar) effects reinforced one another; when they were in opposition the depth effect was much reduced or absent.

Although these considerations favour a relationship between

Fig. 2 Mean data for three observers who saw displays like those illustrated in Fig. 1, but with a 1.0 log unit filter over one eye. When the filter was over the eye that had the extra bar, the percentage of trials on which the observer reported a clear depth effect (ordinate) was large. When the filter and the extra bar were in opposite eyes, there were many fewer confident depth judgments.



the Pulfrich effect and the effect reported here, the latter is not to be explained either by existing knowledge of static stereopsis, or by the conventional explanation of the Pulfrich effect itself. The most nearly related stereoscopic effect is that of "Panum's limiting case", in which a single bar in one eye is fused with a pair of bars in the other eye, to yield an impression of two bars at different distances. This is what will be seen if the two halves of Fig. 1 are fused in a stereoscope. In my display, however, there was not an impression of lines at different distances, but rather of a set of lines moving in the same orbit. It seems, then, that there is an important difference between the dynamic and static versions of the "limiting case". Similarly, Fertsch's classical explanation⁵ of the Pulfrich effect in terms of latencies will not apply here. Fertsch proposed that the filtered eye signalled the actual position of the target at some later time than the unfiltered eye. In my experiments, however, the most advanced (leading) edge of the left and right-eye targets was identical, and differed only in one eye having a longer "trailing" edge. This reinforces other objections that have been advanced against the latency interpretation of the Pulfrich phenomenon⁵. The alternative explanation of filter-induced depth suggested by the present experiment is that the filter causes a trailing edge disparity between the two eyes, and that depth is determined, either by fusion of trailing edges of the signals, or after some spatial averaging has taken place. The possible importance of trailing edge information was realised long ago by Edmund⁶: "Of a movable object, the one eye particularly sees the posterior contour trailing behind, and that is the reason why one thinks that the image of the one eye really succeeds that of the other eye and thus, as Pulfrich's observation shows, receives a false stereoscopic impression."

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Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse

RANDOM X inactivation makes the female mammal a natural mosaic for clones of cells having either the maternally derived X (X^m) or paternally derived one (X^p) which is genetically inactive¹. There are, however, instances in which inactivation is obviously not random²⁻⁷. Non-randomness was inferred from studies made on differentiated cells remote from early embryonic cells in which inactivation occurred. Thus it is not clear whether the randomness of the X inactivation process was influenced or whether cell selection occurring after random inactivation was responsible for the ultimate non-random appearance⁴⁻⁹. In an effort to determine the embryonic stage at which the X chromosome initiates differentiation in female mouse embryos heterozygous for Cattanach's translocation¹⁰, we found that the mosaic composition was consistently biased in extraembryonic membranes, whereas it was not necessarily so in the embryonic body.

Cattanach's translocation comprises an insertion of a segment from chromosome 7 into an X (refs 11 and 12), making the X (X^b) the longest element in the cell¹³. In somatic cells from female heterozygotes, either X^b or the

normal X (X^n) replicates asynchronously with the remaining chromosomes of the complement^{14,15}. This is consistent with the c-variegation in their coats¹⁶. Since asynchronous replication of one X chromosome is a cytological manifestation of genetic inactivity^{16,17}, we used this criterion to ascertain the inactive X in the present study.

The allocyclic X chromosome of the mouse usually becomes late replicating by 8.5 d of gestation¹⁸. Before this stage the same chromosome initiates DNA replication later and finishes it earlier than the rest of the complement¹⁸, which renders replication in the middle of the S phase more active in the former than in the latter. We expected, therefore, that the acridine orange (AO) fluorescence technique after the incorporation of 5-bromodeoxyuridine (BrdU)^{19,20}, for an appropriate period before fixation may allow not only the identification of every autosomal pair but also the distinguishing of the allocyclic X chromosome from the isocyclic one. Preliminary tests, in fact, proved the technique satisfactory; the allocyclic X was identified in more than 80% of metaphases from 7.5-d embryos as the palest red (Fig. 1) or the brightest green element in the complement depending primarily upon the amount of incorporated BrdU²⁰.

The 6.5-d-old embryo consists of two germ layers with minimal mesodermal elements, and the proamniotic cavity and the exocoel join to form an elongated narrow lumen. The whole embryo, being small at this stage, provided just enough material for a single slide. Either X^b or X^n showed differential staining (Fig. 2) in the heterozygous females from reciprocal crosses between the chromosomally normal (A/He) mice and the animals bearing a translocation. Surprisingly, however, the allocyclic X was predominantly paternal (Table 1). The proportion of cells with an allocyclic X^p closely agreed in heterozygous females from both types of crosses. This may imply that the decision of X inactivation was not necessarily random, otherwise the random decision was hidden by the overgrowth of cells having the isocyclic X^m . Before this point is made clear, it seems essential to know the distribution of two types of cells with either the allocyclic X^p or X^m in the embryo. If both cell types distribute unevenly, certain recognisably large areas consisting of a single cell type could be expected. We looked for such specific embryonic regions in embryos at 7.5 and 8.5 d of gestation.

The 7.5-d embryo, at late egg cylinder stage, is divided by the amnion into the embryonic and extraembryonic areas, which were studied separately. The mosaic constitution of both regions proved outstandingly different. The allocyclic X^p was found in less than 70% of cells from the embryonic area, whereas it was found in more than 90% of cells from the extraembryonic area. The decreased overall frequency of cells with the allocyclic X^p at this stage might be attributed to the increase of the embryonic elements relative to the extraembryonic ones in comparison with the earlier stage.

Mosaic composition was studied in five well-defined regions isolated from 8.5-d embryos, the anterior and posterior halves of the embryonic body, the allantois, the yolk sac, and the chorion. Cells with the allocyclic X^p were found to outnumber those with the allocyclic X^m in extraembryonic membranes, whereas the former was only slightly in excess of the latter in the embryonic body as such. The extreme instance was the chorion where the X^p seemed allocyclic in more than 95% of the constituent cells. Since intermingling of cells from different embryonic structures could not be rigorously excluded by the present dissection procedures, it is possible that the chorion is composed exclusively of cells in which X^m is active.

It therefore seems probable that X^p is mostly inactive in the chorion and the yolk sac of embryos heterozygous for Cattanach's translocation. Evidently, we are not dealing with the O^{hv} type mutation which makes the inactivation

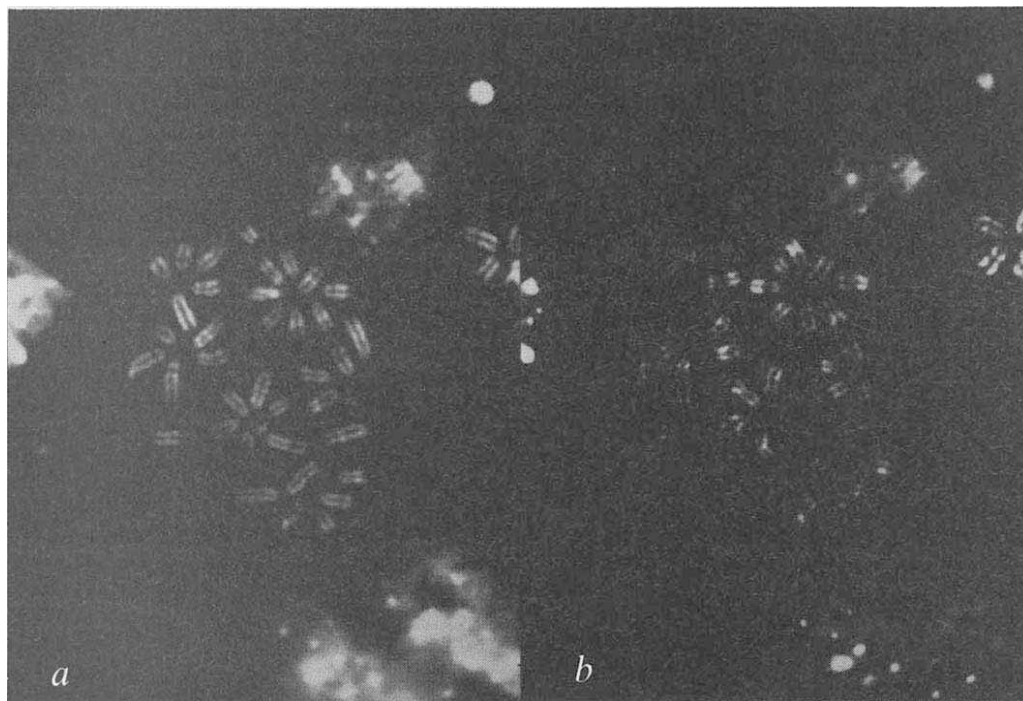


Fig. 1 Verification of the BrdU-AO technique in ascertaining the allocyclic X chromosome in the mouse (T6 heterozygote in this photograph). The brightest (allocyclic) X chromosome after QM technique (a) corresponds to the palest element in the complement after BrdU-AO technique (b). Embryos 7.5 d after fertilisation were taken out of the uterine cavity, incubated at 37 °C for 8 h in Eagle's medium supplemented with foetal calf serum (20%) and BrdU (100 µg ml⁻¹). Colcemide (1 µg ml⁻¹) was added 2 h before collection. Air-dried slides were prepared accordingly to a modified method of Wroblewska and Dyban²¹ as described by Takagi and Oshimura²²

process nonrandom^{23,24} since both cell types were nearly equally represented in the embryonic body as a whole. The slight excess of cells with an allocyclic X^p in the embryonic body seems to be at variance with the lower levels of the c-variegation when X^t is inherited from the father rather than from the mother²⁵. Of particular interest are the mechanisms responsible for accomplishing divergent mosaic compositions in different regions of a single embryo. Our finding is apparently incompatible with random inactivation followed by random sampling of primordial cells for the extraembryonic structures or random inactivation after the divergence of the embryonic from the extraembryonic anlage, unless reversal of inactivation or extensive cell selection is advocated.

It is tempting to postulate that the preferential paternal X inactivation in the extraembryonic membranes is not restricted to Cattanach's translocation, but is common in the laboratory mouse and possibly in some other mam-

malian species. The eutherian type random X inactivation system of gene dosage compensation is considered to have evolved from the marsupial type paternal X inactivation system^{26,27}. If so, does the peculiar feature represent a character directly inherited from the paternal X inactivation system? Or, has it been evolved after the establishment of random inactivation? In either case, it seems highly unlikely that the specific pattern of inactivation has been retained without any advantages for embryogenesis. Thus an assumption could be made that the active X^m in the extraembryonic membranes is favourable, though not indispensable to embryonic development. We did in fact find an exceptional case of a grossly retarded 8.5-d embryo whose yolk sac was composed predominantly of cells with an allocyclic X^p, but further studies are essential for testing the validity of our tentative hypothesis. Investigations along these lines might also prove significant in man.

We wish to thank Dr Mary F. Lyon, MRC, Radiobiology

Table 1 Parental influence on X-inactivation mosaic composition in 6.5–8.5-d-old female embryos carrying Cattanach's translocation

Area examined	Age (d)	Mating type	No. of embryos examined	No. of cells examined	% Cells with allocyclic X ^p (range)
Whole embryo	6.5	I*	19	789	87(71–98)
		II†	22	928	86(77–100)
Embryonic region	7.5	I	11	980	66(53–82)
		II	16	1,729	68(48–80)
Extraembryonic region	7.5	I	11	677	91(83–100)
		II	16	1,094	92(79–100)
Anterior half of embryo	8.5	I	7	552	52(39–65)
		II	6	614	52(30–66)
Posterior half of embryo	8.5	I	7	1,056	55(43–69)
		II	7	774	57(44–70)
Allantois	8.5	I	6	44	77(67–100)
		II	5	179	66(55–83)
Yolk sac	8.5	I	5	745	90(88–98)
		II	7	378	89(52–98)
Chorion	8.5	I	7	444	97(96–100)
		II	7	482	98(95–100)

*Xⁿ/Xⁿ × X^t/Y

†X^t/Xⁿ × Xⁿ/Y

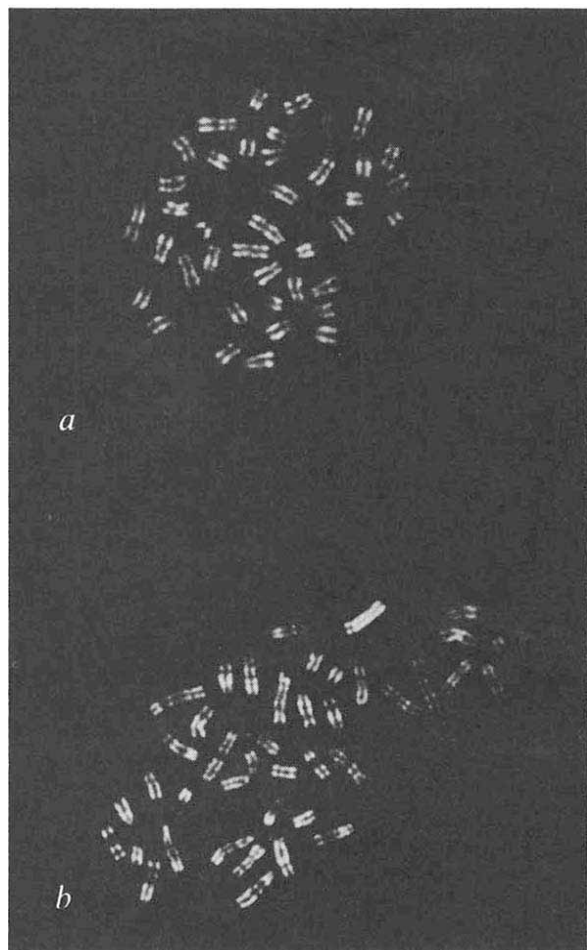


Fig. 2 Differential staining of the allocyclic X chromosome in cells from a 6.5-d-old female embryo heterozygous for Cattanach's translocation. The allocyclic X¹ fluoresced faintly in *a*, whereas the allocyclic X^a fluoresced brightly in *b* depending, most probably, on the relative amount of incorporated BrdU.

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Evidence derived from sister chromatid exchanges of restricted rejoining of chromatid subunits

SISTER chromatid exchanges (SCE) were first described by Taylor *et al.*¹ using autoradiographic techniques. After DNA replication in the presence of tritiated thymidine and subsequent replication in the absence of this radioisotope, two types of SCE were observed in colchicine-induced tetraploid cells. Twin exchanges represented events occurring in the second division cycle whereas single exchanges represented events occurring in the first division cycle.

If SCE rejoining occurs randomly between chromatid subunits, the ratio of twin to single SCE should theoretically be 1:10 (ref. 2). Alternatively, if SCE rejoining is restricted due to inherent polarity, this ratio should theoretically be 1:2 (ref. 2). Experimental support for these alternatives through the use of tritiated thymidine and autoradiography has been inconclusive, with ratios of twin to single SCE varying from 2.4:1 to 1:10 (refs 3 and 4). As an alternative method to examining tetraploid cells, the frequencies of SCE have been compared in metaphase cells one and two division cycles after tritiated thymidine labelling^{5,6}. Again, results have been inconsistent, providing no clear evidence for either random or restricted rejoining of chromatid subunits. This variability is probably related to the use of tritiated thymidine, an agent known to induce SCE⁷.

Recently, techniques have been developed which make possible the examination of SCE without radioactive labelling. These techniques are based on the differential staining of sister chromatids by fluorescent compounds^{8,9} or Giesma¹⁰ after a minimum of two division cycles in the presence of bromodeoxyuridine (BrdU). It was, therefore, decided to use these new techniques to re-examine whether SCE rejoining is random or restricted by comparing the frequency of SCE occurring in the first two division cycles with SCE occurring in the third division cycle.

Lymphocyte cultures from ten male subjects were incubated at 37 °C for 72 h in total darkness using culture media containing phytohaemagglutinin-M (Gibco) and 0.9 µg ml⁻¹ BrdU. Colcemid (Gibco) was added to a final concentration of 0.1 µg ml⁻¹ for the final 3 h of incubation. The cells were collected, fixed and slides prepared.

Metaphase chromosomes from cells having passed through one division cycle in the presence of BrdU exhibit no differential sister chromatid staining with acridine orange. Consequently, SCE cannot be detected (Fig. 1*a*). After two BrdU cell cycle divisions (Fig. 1*b*), SCE can be clearly observed since acridine orange stained chromatids unifilarly substituted with BrdU fluoresce more intensely than bifilarly substituted chromatids⁹. Mitotic cells after three BrdU cell cycle divisions can be easily distinguished from cells having undergone two replications in the presence of BrdU since only one-fourth of total sister chromatid length retains thymidine and fluoresces brightly (Fig. 1*c*).

In the examination of SCE frequency, previous investigators^{5,12} have avoided the inclusion of SCE which seem to involve the centromeric region. Assumed difficulty in distinguishing centromeric SCE from chromosomal twists in this region was the basis for this exclusion. Centromeric SCE occurring in the first two division cycles, however, can be unequivocally determined in third division metaphase cells where they appear as chromosomes with only one arm brightly fluorescent (Fig. 1*c*). Examination of centromeric SCE fre-

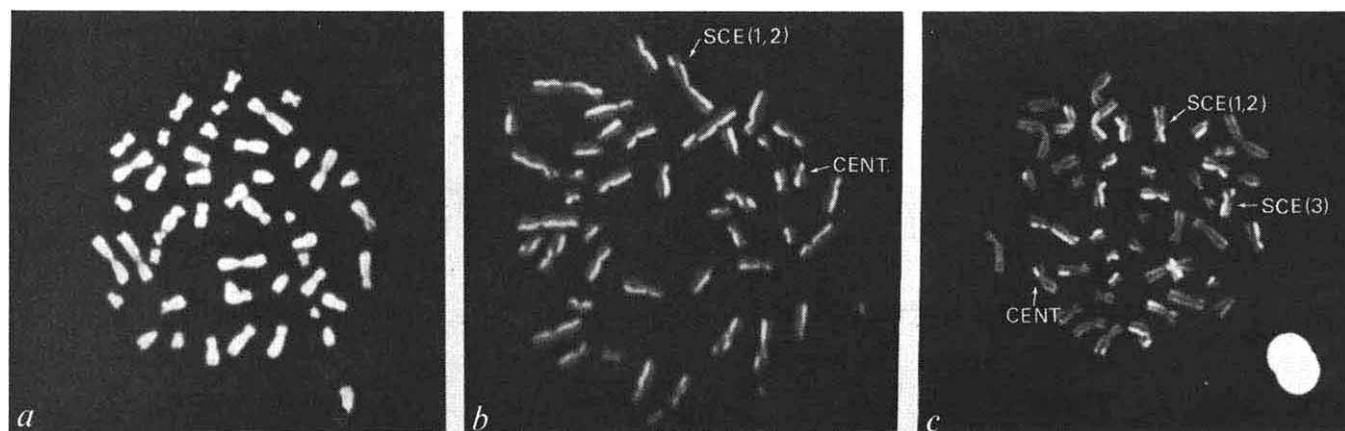


Fig. 1 Fluorescence photomicrographs of metaphase chromosomes. *a*, Cell after one division cycle in BrdU with all chromatids unifilarly substituted with BrdU and therefore no differential fluorescence; *b*, cell after two division cycles in BrdU with one-half of all sister chromatid regions bifilarly substituted with BrdU. These regions fluoresce less intensely than unifilarly substituted sister chromatid regions; *c*, cell after three division cycles in BrdU with only one-fourth of all chromatid regions still unifilarly substituted with BrdU. Arrows with (CENT.) denote centromeric SCE, arrows with (SCE (1, 2)) denote SCE which had occurred within the first two division cycles, arrows with (SCE (3)) denote SCE occurring in the third division cycle. Heparinised whole blood was cultured in Eagles MEM with Earle's salts, non-essential amino acids, 2 mM L-glutamine, penicillin (10,000 U ml⁻¹), streptomycin (10,000 µg ml⁻¹) (Gibco), and 10% heat-inactivated foetal bovine serum (Flow). Cultures were incubated at 37 °C for 72 h in total darkness in the presence of phytohaemagglutinin-M (Gibco) and 0.9 µg ml⁻¹ BrdU. Colcemid (Gibco) was added to a final concentration of 0.1 µg ml⁻¹ for the final 3 h of incubation. The cells were collected, fixed, and slides prepared²¹. Slides were stained for 5 min in 0.125 mg ml⁻¹ acridine orange⁹ dissolved in phosphate buffer (pH 7.4), rinsed briefly with distilled water, incubated for 10 min in phosphate buffer (pH 7.4) and mounted in the same phosphate buffer. Chromosome fluorescence was observed in a Leitz Orthoplan microscope equipped with epi-illumination from a 200 W mercury light source, BG12 and TK510 dichroic excitor filters, K515 and K510 suppressor filters. Photographs were taken using a ×54 objective.

quency in these cells showed them to occur as a relatively high proportion of all SCE. Consequently, in any comparison of SCE frequencies, it was decided that centromeric exchanges should be included. To assess our ability to distinguish centromeric SCE from chromosomal twists, we examined both second and third division metaphase cells for the proportion of centromeric SCE which occurred in the first two division cycles. Unequivocal centromeric SCE measured in third division metaphase cells accounted for 18% of all SCE occurring in the first two division cycles, whereas centromeric exchanges measured in second division metaphase cells accounted for 21% of all SCE. Therefore, it seems possible to distinguish true centromeric SCE occurring during any cell cycle from apparent chromosomal twists. These values for the proportion of SCE which occurs in the centromeric region are comparable with previously reported estimates^{5,13}.

The total SCE frequencies occurring in the first two division cycles are listed in Table 1. No significant difference was observed between measurements made in second or third division metaphase cells. Therefore, these data were combined and the ratio of SCE occurring in the first two division cycles to SCE occurring in the third division cycle was calculated. If SCE occur with equal frequency in each division cycle, the expected frequency of SCE occurring in the first two division cycles should be twice the frequency of SCE occurring in the third division cycle. Since only half of the SCE occurring in the third division cycle can be detected (half of the SCE occur in chromo-

somal regions completely substituted with BrdU), the expected ratio of the first two division cycle SCE to third division cycle SCE becomes 4 : 1. The derived ratio of 4.05 : 1 is clearly consistent with restricted rejoining of sister chromatid subunits. The ability to attain such a close approximation to the predicted ratio is most likely a result of the use of this new non-radioactive technique.

It has not been completely resolved whether the SCE observed with this technique are spontaneous or induced by BrdU. High concentrations of BrdU¹⁰, or low concentrations of BrdU in the presence of light¹⁴, have been shown to induce SCE. It is not clear whether BrdU at the low concentration we used in the absence of light induces SCE^{10,15}. Irrespective of whether the observed SCE were induced or spontaneous, our results indicate that the observed SCE clearly involved both chromatid subunits with restrictions on rejoining due to subunit polarity. This does not exclude the possibility that in radiation or chemically treated systems, a proportion of induced SCE could be the result of exchange between single chromatid subunits.

Although our data strongly support a mononeme structure for eukaryotic chromosomes (that is, composed of one DNA duplex with chromatid subunits being single polynucleotide strands), it is also possible to devise a model by which SCE can occur with polarity restrictions on rejoining between sister chromatids composed of more than one DNA duplex. Such a polyneme model for SCE rejoining would, however, be several orders of magnitude more complex than a mononeme model.

Table 1 Mean SCE frequency

Division cycles in BrdU	(a)	(b)	Derived ratio a/b	Expected* ratio a/b
	Frequency of SCE occurring in the first two division cycles (mean ± s.e.)	Frequency of SCE occurring in the third division cycle (mean ± s.e.)		
2	10.50 ± 0.31 (165)	—	—	—
3	10.05 ± 0.33 (165)	2.54 ± 0.15 (165)	3.96	4.00
2 and 3	10.28 ± 0.23 (330)	2.54 ± 0.15 (165)	4.05	4.00

Thirty or more metaphase cells per individual (one-half second metaphase cells, one-half third metaphase cells) were optically examined by two observers for SCE frequency. Number of cells examined are shown in parentheses.

* Expected ratio based on restricted rejoining of chromatid subunits would be 2 : 1. Since only half of all SCE occurring in the third division cell cycle can be detected, however, the expected ratio becomes 4 : 1.

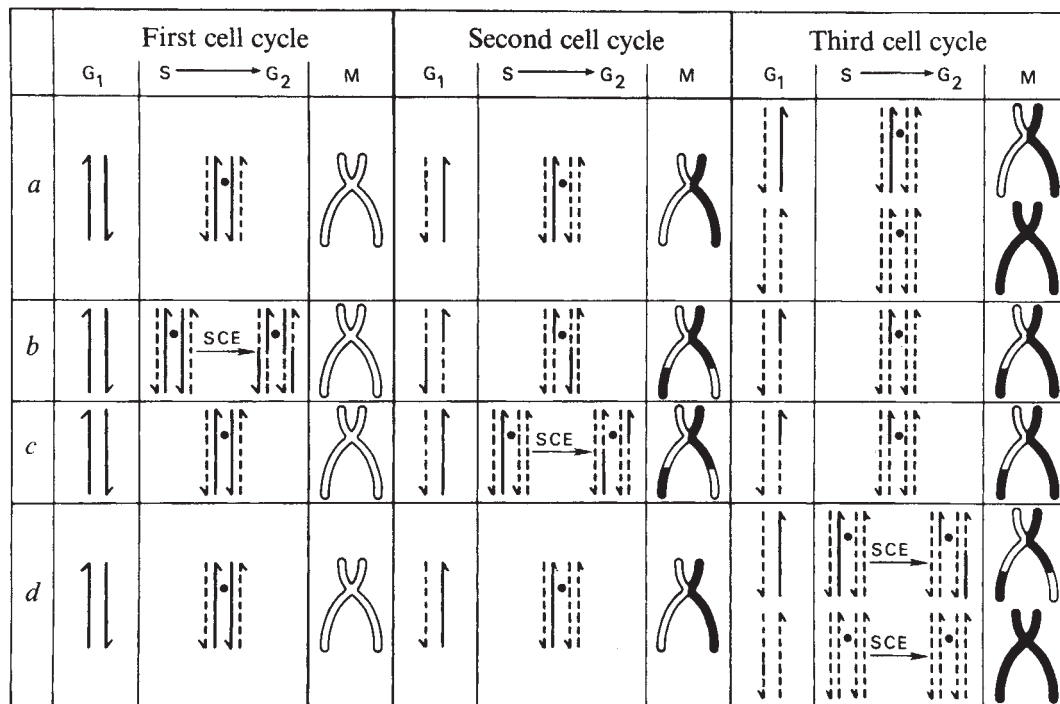


Fig. 2 Progression of cells through three division cycles in the presence of BrdU based on the mononeme model of eukaryote chromosome structure. *a-d*, Appearance of chromosomes during three division cycles with: *a*, no SCE occurring; *b*, SCE occurring in the first division cycle; *c*, SCE occurring in the second division cycle; *d*, SCE occurring in the third division cycle. —, Thymidine; ----, BrdU-bearing DNA strand. Direction of arrow indicates polarity of chromatid subunits. Black chromosomal regions indicate regions which fluoresce less intensely with acridine orange. White chromosomal regions indicate regions which fluoresce more intensely with acridine orange.

Figure 2 is a schematic representation of the results of SCE occurring in each of the three successive replication cycles in the presence of BrdU based on the mononeme model of eukaryotic chromosome structure.

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Genes for immunoglobulin heavy chain and serum prealbumin protein are linked in mouse

SEVERAL cases of allelic variation have been reported in the mouse that are presumably determined by genes for the variable region of the heavy chain of immunoglobulins¹⁻⁸. These genes control qualitative and/or quantitative differences in the immune response to particular antigens, and exhibit close linkage to the cluster of genes (*Ig-1*, *Ig-2*, *Ig-3*, *Ig-4*) that determine the allotypic differences residing in the constant portion of the heavy chains of several immunoglobulin classes⁹⁻¹¹. (No

recombinants have been observed among the latter in over 2,000 backcross mice.) Significantly, several presumptive recombinants have been recovered that seem to separate some variable region genes from the allotype loci, and some variable region genes from each other^{1,12}. Outside genetic markers would be very valuable in identifying and interpreting these presumptive recombinants. Unfortunately, efforts to place the heavy chain allotype locus in the mouse linkage map have been unsuccessful^{9,10,13}. We report here the discovery of close linkage between *Ig-1* and the serum prealbumin locus of the mouse.

The autosomal prealbumin locus (*Pre*) determines the presence or absence of a minor serum protein band that migrates ahead of the albumin band on electrophoresis¹⁴. Density of the band is hormone dependent, and is greatest in sexually mature males. The *Pre*^a allele determines the presence of the band in both homozygotes and heterozygotes, whereas *Pre*^o/*Pre*^o homozygotes lack the prealbumin band. The absence of a detectable prealbumin band in *Pre*^o/*Pre*^o mice may reflect an altered mobility that coincides with the albumin fraction. The nature, function, and origin of the prealbumin protein is unknown^{14,15}. The *Pre* locus has not been located in the linkage map¹⁴.

Evidence favouring linkage of *Ig-1* and *Pre* came initially from observing the joint segregation of these loci in recombinant inbred (RI) lines. Three different sets of RI lines were studied. CXB lines developed by Bailey were derived by brother-sister mating from the F₂ generation of the cross of C57BL/6By and BALB/cBy (ref. 16). AKXL and BXD sets were similarly produced by Taylor from the crosses of AKR/J × C57L/J (ref. 17) and C57BL/6J × DBA/2J (ref. 18) respectively. It is clear that unlinked genes will be randomised in the F₂ generation, whereas closely linked genes will tend to become fixed in the same combinations as they entered the cross.

The results of typing the three sets of RI lines for *Ig-1* and *Pre* are presented in Table 1 with the genotypes of the progenitor strains. A preponderance of parental genotypes with respect to *Ig-1* and *Pre* was observed. Out of 54 independently derived RI lines, only 14 exhibited recombinant phenotypes (*P* < 0.001). If the loci were unlinked, a 1:1 ratio of parental to recombinant genotypes would be expected.

To confirm the linkage, sexually mature males from the C57L/J × (AKR/J × C57L/J)F₁ backcross were typed both for the presence of the prealbumin band and the AKR/J allotype. The numbers of mice in each of the four classes were as follows: *Pre*^a/*Pre*^o, *Ig-1*^a/*Ig-1*^a (51); *Pre*^o/*Pre*^o, *Ig-1*^a/*Ig-1*^a (54);

Pre^a/Pre^o , $Ig-1^a/Ig-1^a$ (9); Pre^o/Pre^o , $Ig-1^d/Ig-1^a$ (5). These results differ significantly from the expected 1:1:1:1 ratio in favour of linkage ($P < 0.0001$). The estimated recombination fraction from the backcross data is 0.118 ± 0.034 .

During the successive generations of inbreeding in the formation of an RI line, there are multiple opportunities for recombination between linked loci. The probability of fixing a recombinant genotype (R) is $4r/(1 + 6r)$, where r is the probability of recombination in a single meiosis¹⁹. Substituting 14/54 for R provides an estimate for r of 0.106 ± 0.040 . The authors thank Dr E. L. Green for pointing out that r is equal to $R/(4 - 6R)$ and that the large sample variance of r estimated this way is $r(1 + 2r)/(1 + 6r)^2/4N$, where N is the number of RI lines. The estimate obtained in this way is very similar to the estimate obtained in the backcross. The combined estimate of r is 0.114 ± 0.024 . As this is a relatively short distance, we are probably justified in equating recombination frequency with map distance. The estimated map distance between Pre and $Ig-1$ is 11.4 ± 2.4 centimorgans (cM). Note that the estimate from the RI lines reflects the average recombination frequency in the two sexes, whereas the backcross data reflect recombination in males only.

Shreffler place the Pre^a allele of CBA/J on to the C57BL/10JSf genetic background by 10 backcrosses followed by brother-sister mating. The strain is designated B10.PRE. The allotype of the line proved to be that of the background strain ($Ig-1^b$) rather than that of the donor strain ($Ig-1^a$), indicating that a crossover in the $Ig-1-Pre$ region had occurred at some stage. This congenic strain may prove to be useful for crossing with Pre^o strains, when the C57BL background, the $Ig-1^b$ allele, and the Pre^a allele are all desired. It should also be useful in studies aimed at characterising the prealbumin protein as to site of synthesis, turnover rate and function.

The results of typing five $Ig-1$ congenic strains^{1,10,11,20} in which the $Ig-1$ alleles from strains C57BL/Ka and AL/N (both Pre^o) were placed on the BALB/c (Pre^a) background are

shown in Table 2. The BAB/14, C.B17, and C.B26 strains are all Pre^o type like the donor C57BL/Ka strain, indicating that no crossover has occurred between $Ig-1$ and Pre during the backcrossing and inbreeding process. This provides further confirmation of the linkage between $Ig-1$ and Pre . The C.AL9 and the C.AL20 strains are both Pre^a type unlike the donor AL/N strain, indicating that a crossover occurred between $Ig-1$ and Pre during their development. These results plus the finding of crossing over in the B10.PRE congenic strain, provide a basis for a third estimate of the recombination frequency between $Ig-1$ and Pre . The likelihood of obtaining recombination in the B10.PRE and C.AL series, without recombination in the C.B series is maximised by a recombination frequency of about 0.05, a value similar to the estimates obtained from the RI lines and backcross data. Note that the BAB/14 strain is an apparent recombinant between $Ig-1$ and the closely linked gene that determines an idiosyncrasy specific immune responsiveness to α -1,3 dextran¹. This implies the gene order: $Pre-Ig-1-V_H-DEX$, unless a double crossover is postulated.

Extensive typing of the three sets of RI lines at other loci has not revealed the chromosomal location of Pre and $Ig-1$. The mapped loci with their respective chromosome numbers in parentheses, for which there exists at least partial typing in the CXB, AKXL, and BXD RI lines, are as follows: CXB: a (2), $Mup-1$ b $H-15$ $H-16$ $Gpd-1$ (4) $H(go)$ (5), $H-22$ $Gpi-1$ c Hbb $H-1$ (7), $Es-1$ $H-19$ (8), $Mod-1$ $H-7$ $Fv-2$ Bgs (9), Hba (11), and $H-2$ (17); AKXL: ln $Dip-1$ ald (1), $H-3$ Svp (2), $Mup-1$ b $Gpd-1$ (4), Gus (5), $Ly-2$ (6), c Hbb (7), $Es-1$ (8), $Thy-1$ $Fv-2$ (9), $Es-3$ (11), $H-2$ (17); BXD: $Id-1$ $Dip-1$ (1), Svp (2), $Mup-1$ b $Gpd-1$ $Fv-1$ (4), $Pgm-1$ (5), $Ly-2$ (6), $Gpi-1$ Hbb (7), $Es-1$ (8), d $Mod-1$ $Fv-2$ Bgs (9), $Es-3$ (11), and $H-2$ (17).

We have typed for prealbumin a number of inbred strains maintained by the Jackson Laboratory. Knowledge of this typing may be helpful in planning linkage studies of this region. To the list of Pre^a -bearing strains reported by Shreffler¹⁴ (AKR/J, BALB/cJ, CBA/J, DBA/1J, DBA/2J, RF/J, and WB/Re),

Table 1 Segregation of $Ig-1$ and Pre among three sets of RI lines

RI lines (and progenitor strains)*	Genotypes†		No. of parental phase RI lines	No. of recombinant phase RI lines
	$Ig-1$	Pre		
(BALB/cBy), CXBG, CXBJ	a	a	2	
(C57BL/6By), CXBD, CXBE, CXBI	b	o	3	
CXBH, CXBK	b	a		2
(AKR/J), AKXL-8, 12, 17, 23, 25, 28, 37, 38	d	a	8	
(C57L/J), AKXL-1, 4, 6, 11, 16, 19, 21	a	o	7	
AKXL-24	d	o		1
AKXL-13, 14, 18, 29, 36	a	a		5
(C57BL/6J), BXD-1, 2, 3, 5, 13, 14, 21, 22, 23, 29	b	o	10	
(DBA/2J), BXD-6, 11, 15, 16, 17, 20, 24, 27, 28, 30	c	a	10	
BXD-8, 9, 19, 25	b	a		4
BXD-12, 18	c	o		2
Totals				
Observed			40	14
Expected			27	27

$$\chi^2_1 = 12.5, P < 0.001$$

*CXB RI strains were sib-mated more than 30 generations before testing. AKXL and BXD RI lines were tested at a time when they were incompletely inbred ($F > 0.83$). Lines that were known to be segregating at either $Ig-1$ or Pre are not included. Certain deviations from continuous sib-mating occurred in AKXL lines 6, 11, and 16, the net effect being to slightly increase the probability of fixing C57L/J alleles. Several of the AKXL and BXD RI lines are now extinct, and other lines will likely become extinct.

†CXB RI strains were $Ig-1$ typed by Dr M. Potter²³. AKXL and BXD RI lines were tested for the presence of the $Ig-1^a$ and $Ig-1^b$ alleles, respectively. Thus, from a d (or b) designation of RI lines for $Ig-1$, it should be interpreted that the $Ig-1^a$ (or $Ig-1^b$) allele was present in the line at the time it was tested. (Requisite antisera to test for the presence of $Ig-1^a$ and $Ig-1^b$ alleles were not available.) Allotyping was carried out using immunodiffusion with antisera made in BALB/c mice against conjugates of *Bacillus pertussis* and anti-pertussis antibodies made in NZB ($Ig-1^c$, cross reactive with $Ig-1^d$) and BAB/14 ($Ig-1^b$, otherwise congenic to BALB/c) mice, respectively. These allotyping sera precipitated only with immunoglobulins of appropriate strains⁹⁻¹¹.

Serum samples from two to six sexually mature males of each RI line were tested for the presence of prealbumin protein. Electrophoresis of fresh or frozen sera was carried out using a vertical gel electrophoresis cell (EC470, EC Apparatus, St Petersburg, Florida). A 5% Cyanogum 41 acrylamide gel was prepared as described by the manufacturer. Buffer was 0.09 M Tris, 0.09 M borate, 2.5 mM EDTA, pH 8.4. Electrophoresis of 10–20 μ l serum samples was carried out for 2 h at a potential of 250 V. Gels were stained for 1 h with 1% Amido Black in 5:5:1 methanol-water-acetic acid solution, and destained in the same solvent.

Table 2 Prealbumin types of several congenic strains in which different *Ig-1* alleles have been placed on the BALB/c (*Ig-1^a*, *Pre^a*) background by serial backcrossing*

Congenic strain	Donor strain	Generations of backcrossing	Crossover?
BAB/14 (<i>Ig-1^b</i> , <i>Pre^a</i>)	C57BL/Ka (<i>Ig-1^b</i> , <i>Pre^a</i>)	14	No
C.B17 (<i>Ig-1^b</i> , <i>Pre^a</i>)	C57BL/Ka (<i>Ig-1^b</i> , <i>Pre^a</i>)	17	No
C.B26 (<i>Ig-1^b</i> , <i>Pre^a</i>)	C57BL/Ka (<i>Ig-1^b</i> , <i>Pre^a</i>)	26	No
C.AL9 (<i>Ig-1^d</i> , <i>Pre^a</i>)	AL/N (<i>Ig-1^d</i> , <i>Pre^a</i>)	9	Yes
C.AL20 (<i>Ig-1^d</i> , <i>Pre^a</i>)	AL/N (<i>Ig-1^d</i> , <i>Pre^a</i>)	20	Yes

*The *Ig-1* congenic strains described here as well as the AL/N strain are maintained at the Institute for Cancer Research. The BAB/14 and C.B17 congenic strains were separated at the 13th backcross generation. Brother-sister inbreeding was instituted after one and four additional backcrosses in the BAB/14 and C.B17, respectively. The C.B26 congenic strain was separated from the C.B17 strain at the 17th generation. The C.B26 strain was backcrossed nine additional generations before inbreeding. The first 13 backcrosses were to the BALB/cAn strain. Subsequent backcrossing in the C.B17 and C.B26 strains was to the BALB/cAnN1cr subline. The C.AL9 and C.AL20 strains are related to each other in the same way that C.B17 and C.B26 are related. In the C.AL series all backcrosses were to the BALB/cAn strain. The designations used to represent these congenic strains varies between publications and do not follow the rules for standardised nomenclature. The designations used here are intended to be descriptive rather than definitive.

the following strains may be added: BDP/J, BUB/BnJ, CE/J, I/LnJ, LT/Re, NZB/BINJ, P/J, RIII/2J, SEA/GnJ, SEC/1ReJ, SJL/J, ST/bJ, and 129/J. To the list of *Pre^a*-bearing strains reported by Shreffler (A/HeJ, A/J, C57BL/6J, C57BL/10J, C3H/HeJ, and WC/Re), the following strains may be added: AU/SsJ, CBA/CaJ, C3HeB/FeJ, C57BL/KsJ, C57BL/10Sn, C57BR/cdJ, C57L/J, C58/J, HRS/J, LG/J, LP/J, MA/J, MWT/Ww, SM/J, SWR/J, and WK/Re. The difference between the CBA/J and CBA/CaJ sublines explains a discrepancy in the reports of Shreffler¹⁴ and Reuter *et al.*¹⁵. The latter apparently typed a CBA subline related to CBA/CaJ. These two sublines exhibit multiple differences²¹, indicating that they were either incompletely inbred at the time of separation or that contamination occurred. We have been unable to confirm Shreffler's *Pre^a* typing of the WB/Re strain.

The *Pre* locus described by Shreffler and considered here should not be confused with another prealbumin electrophoretic variant designated *Pre-1* described by Claxton *et al.*²² that segregates independently of Shreffler's *Pre* locus. The close linkage of the *Pre-1* locus to the brown (*b*) locus in chromosome 4 suggests that it is identical to the *Mup-1* locus²³ that determines an electrophoretic variant in major urinary protein which is also detectable in serum¹⁵.

The *Pre* locus has several merits as a marker for the *Ig-1* region; fresh or frozen serum is used for typing; there is a significant polymorphism among the commonly used inbred strains; typing is relatively easy, inexpensive and reliable. The weak and variable expression of the prealbumin gene in females and the inability to reliably distinguish between *Pre^a*/*Pre^a* and *Pre^a*/*Pre^o* mice limits the usefulness of this marker^{14,15}. Both of these limitations may be removed by refinements in the typing procedure.

In man the heavy chain allotype locus (*Gm*) is linked to the protease inhibitor locus (*Pi*) with a map distance of about 25 centimorgans²⁴. The *Pi* locus comprises an allelic series of electrophoretic variants of the serum glycoprotein α_1 -anti-trypsin²⁵. Although murine α_1 -anti-trypsin seems to share some chemico-physical characteristics with prealbumin^{15,26}, the former is electrophoretically monomorphic among inbred strains²⁶. Further biochemical and biological characterisation of the prealbumin protein is in progress.

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Chemotaxis of lymphoblasts

LYMPHOCYTES are motile cells whose movement *in vivo* seems to be directed, yet investigators over many years have repeatedly failed to show chemotaxis of lymphocytes *in vitro*. There have been some recent reports that lymphocytes migrate into micro-pore filters towards substances placed below the filter¹⁻³, but this migration was not found to be chemotactic³. It seemed to us that our and other people's failure to demonstrate lymphocyte chemotaxis might be because we were using the wrong population of cells. The lymphocytes which can be shown most clearly to migrate into inflammatory lesions *in vivo* are blast-transformed cells⁴⁻⁶, and we therefore examined the migration in Boyden chambers of lymphoblasts from two sources; firstly cloned human lymphoblast cell lines maintained in continuous culture⁷; and secondly blast cells from the lymph nodes of CBA mice, either without deliberate sensitisation with antigen, or following exposure to the contact sensitising agent, oxazolone. Both human and mouse lymphoblasts were shown to migrate into filters towards chemoattractants. The nature of this migration is discussed below.

Locomotion and chemotaxis of blast-transformed lymphocytes were measured using the micropore filter technique as described previously⁸. For mouse lymphoblasts a filter of 8 μ m pore size was used. Because the human blasts were larger, filters of 12 μ m pore size (Sartorius, Göttingen, Germany) were used. The distance migrated by the leading front of cells was measured as described by Zigmond and Hirsch⁹ after 3 h. At this time, the leading-front cells in a population of lymphoblasts migrating towards a strong chemoattractant have

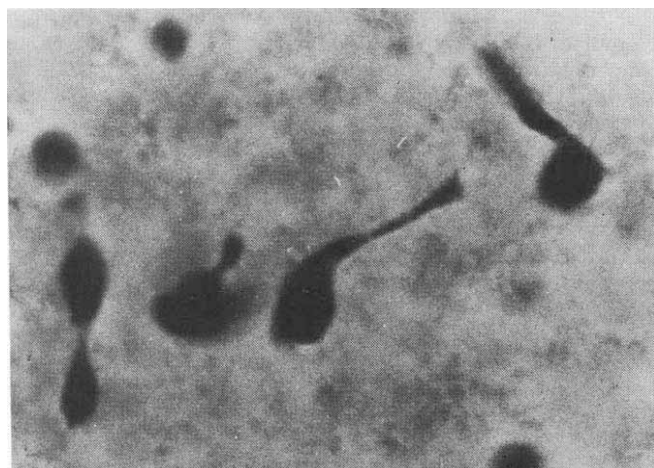


Fig. 1 Human lymphoblasts (line SMI₄) migrating in a micropore filter and showing locomotor morphology. The picture is taken from above and the cells are at a depth of 60 μm in the filter. ($\times 900$)

migrated deeply into the filter but not reached the lower surface. This compares with 2 h for human blood monocytes migrating the same distance in the same filters. The cells did not require to be incubated in CO_2 (refs 1, 2) nor was prolonged incubation (18 h) necessary as has been suggested previously¹. Table 1 shows the migration of lymphocytes from seven human lymphoblast cell lines in Gey's solution alone and in the presence, below the filter, of substances previously shown to be chemotactic for monocytes or neutrophils. The cell lines varied considerably in their random unstimulated migration and in their response to chemoattractants. Cells from all of the lines migrated further in the presence of endotoxin-activated plasma than in its absence and most of them also showed an enhanced response to casein. The migrating cells showed a typical locomotor morphology with a broadened leading edge and a narrow tail which occasionally became very elongated (Fig. 1).

To test whether the migration was chemotactic, experiments were set up in which the absolute concentration and the gradient of the chemoattractant were varied above and below the filter. The influence of chemoattractants on either rate of locomotion or on its orientation has been distinguished in this way⁹. Table 2 shows that the rate of locomotion of lymphocytes in the absence of a gradient (figures along the diagonal from upper left to lower right) varies with chemoattractant concentration, that is the cells show chemokinesis under these conditions. Furthermore, lymphocytes migrating in a positive gradient penetrated deeper into the filter than would be expected on the basis of concentration-dependent random migration alone; conversely,

lymphocytes migrating in a negative gradient did not penetrate as deeply as would be expected by random migration alone. These results support the conclusion that the locomotor response of human lymphoblasts to activated plasma depends not only on the absolute concentration of the plasma but also on the concentration gradient and is, therefore, chemotactic. Similar results were obtained with three other human lymphoblast lines. Table 2b shows the effects of colchicine on the migration of the same cells. Colchicine enhances concentration-dependent random migration in the absence of a gradient (figures on the diagonal), but colchicine-treated cells lose the capacity to detect and respond to a gradient. Similar results were obtained with vinblastine (10^{-5} M). These results resemble our unpublished data on the effects of colchicine and vinblastine on neutrophil and monocyte locomotion. They support the hypothesis¹⁰⁻¹² that the integrity of microtubules is required for the orientation of locomotion of these cell types but not for locomotion itself.

The lymphocytes from the auricular nodes of unsensitised mice are mostly small cells and the type of filter used was not suitable for the study of the migration of such cells. The small number of blast cells present in suspensions from normal mouse lymph nodes were, however, shown to migrate into filters and gave a dose response to chemoattractants which resembled that of human lymphoblasts (Fig. 2). Cell suspensions made from the draining nodes of mice 72 h after contact sensitisation by painting the ears with oxazolone contained many more blast cells (30%) than suspensions from unsensitised

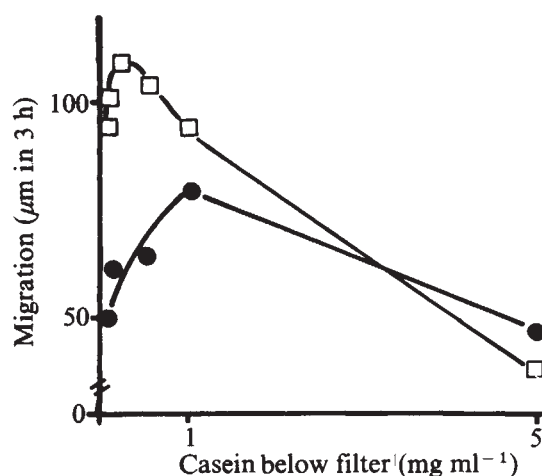


Fig. 2 Migration towards varying doses of casein of lymphoblasts from normal mouse auricular lymph nodes (●) and auricular lymph nodes 72 h after sensitisation with oxazolone (□)

Table 1 Migration of cultured monoclonal human lymphoblasts

Cell line	Source (refs 7, 13, 14, 15)	Ig release into supernatant (ref. 15)	Gey's	Migration (μm in 3 h) towards	Casein 1 mg ml^{-1}
				Endotoxin-activated human plasma (10%)	
SMI ₄	Healthy cord blood	ML	28	98	75
BAR ₁ 1448a	Healthy XXY	GMKL	18	38	57
ANA ₂	Healthy cord blood	GMKL	45	83	—
F ₈₉ 9489	Subacute lymphatic leukaemia	no Ig	21	78	30
FAL ₁ 8440	Healthy cord blood	No Ig	43	72	38
JIM ₁	Healthy adult blood	AMK	90	120	97
ORI ₁	Healthy adult blood	G	17	44	46

The cell lines were obtained from Dr Judith Evans, MRC Clinical and Population Genetics Unit, Western General Hospital, Edinburgh. They were established and maintained in tissue culture as described by Steel⁷. For studies of locomotion they were maintained in Eagle's medium + 10% foetal calf serum and HEPES buffer (3% of a molar solution) changed every 4 d. They were incubated in 5% CO_2 at 37 °C until required for use when they were washed twice in serum-free Gey's solution before being placed in chemotaxis chambers at $2 \times 10^6 \text{ ml}^{-1}$. Normal human plasma was activated by incubation at 37 °C for 30 min with *Shigella flexneri* lipopolysaccharide.

Table 2 The effect of varying concentration gradient and absolute concentration of chemoattractant on locomotion of human lymphoblasts (line F₈₉ 9489)

Lymphocyte migration (μm in 3 h). Mean of 5 fields in each of 2 filters					
a, No colchicine					
	% Endotoxin-activated plasma below filter				
	0	0.5	3.5	6.5	9.5
0	18				
0.5		26	72(52)	81(52)	82(58)
3.5			29(52)	79	85(83)
6.5				67(83)	87
9.5					93(81)
					53(82)
					76(84)
					75(81)
					75
b, Colchicine (10^{-4}M) both sides of filter					
	0	0.5	3.5	6.5	9.5
0	21				
0.5		83	90(91)	96(94)	87(95)
3.5			92(91)	99	93(98)
6.5				89(97)	92(98)
9.5					97
					94(98)
					94(97)
					90(98)
					92(98)
					98

Figures along the diagonal from upper left to lower right indicate distance migrated (μm) in increasing concentrations of the activated plasma but in the absence of a concentration gradient. Above the diagonal, the cells were moving in a positive gradient, below, in a negative one. The figures in brackets are estimates of what migration would have been in each of the tests assuming that the cells detected only the absolute concentration of the chemoattractant but not the gradient. They were calculated after Zigmond and Hirsch⁸. *P* for gradient effect on migration of cells without colchicine <0.0005 ; with colchicine, >0.1 .

nodes (3%) and these cells were much more motile than those from the unsensitised nodes. They usually, but not always, showed considerable random motility in the absence of any chemoattractant (Fig. 2). This was also true of the blast cells from such nodes which did not adhere to nylon wool. Their response to chemoattractants was variable. Typically those which showed high unstimulated motility showed little further increase in motility when placed in the presence of a chemoattractant (Fig. 2). Experiments on the lines of Table 2 were done to test whether oxazolone-stimulated blast cells show a chemotactic response. In two out of three such experiments—the third gave an equivocal result—lymphoblasts from oxazolone-sensitised lymph nodes failed to show evidence of chemotaxis when tested under varying gradient conditions as in Table 2. These cells also consistently showed little or no change in the rate or the direction of their migration after treatment with colchicine. This might suggest that the blast cell population obtained after contact sensitisation shows a form of locomotion which is not directed or restrained by the action of microtubules.

These preliminary results suggest that populations of blast-transformed lymphocytes vary considerably in their locomotor response to chemoattractants, more so indeed than neutrophil or macrophage populations. The majority of cultured human lymphoblastoid B cell lines show low unstimulated motility, move faster in the presence of a chemoattractant, and show variations in their migration when the concentration gradient is varied which are consistent with a chemotactic response. This should be confirmed by time-lapse studies of the migration

of single cells and the demonstration of oriented movement in a gradient by direct observation. Oxazolone-sensitised mouse lymphoblasts, however, show considerable unstimulated migration, and although they also respond to chemoattractants, this response does not seem to be chemotactic. It remains to be established whether these variations in locomotor behaviour reflect fundamental differences between B and T cell populations. It seems likely that the migration of lymphoblasts which we have observed *in vitro* is related to the migration of similar cells *in vivo* into sites of inflammation, especially since activated plasma is similar to attractants found *in vivo* in inflammatory tissue. Whether other examples of apparently directed lymphocyte migration *in vivo* are mediated by chemoattractants remains to be seen.

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Ultrastructural evidence for ectoglycosyltransferase systems

CONSIDERABLE biochemical evidence suggests that glycosyltransferases, in addition to being present in the Golgi apparatus¹, are also located on the outer surface of the plasma membrane of a variety of normal and malignant cell types²⁻⁹. The enzymes catalyse the transfer of specific monosaccharide units from nucleotide sugar donors to appropriate glycoprotein or glycolipid acceptors. Presumably those in the Golgi play a major role in the biosynthesis of glycoproteins and glycolipids¹ while those at the plasma membrane (ectoglycosyltransferases) have been implicated in mechanisms for cell to cell adhesion, recognition and communication²⁻⁷; cell surface repair⁸ and for binding circulating oligosaccharides or glycoconjugates⁹. Although the biochemical evidence for the existence of ectoglycosyltransferases is compelling, it is not definitive. In this report, we use electron microscope (EM) autoradiography to demonstrate the plasma membrane localisation of a N-acetylneuraminic acid ectoenzyme system (ectosialyltransferase, EC 2.4.99.1; the enzyme together with cell surface acceptor molecules constitute the ectoenzyme system⁶). The results provide the first ultrastructural evidence for the presence of such a glycosyltransferase system at a cell surface.

Murine leukaemic L-1210 cells, shown previously in our laboratory⁸ by biochemical methodology to possess galactosyl- and sialyltransferase activities at their external cell surface were again used in this study. The cells were obtained from the ascites fluid of DBA/2 female mice, washed twice in ice-cold phosphate buffered saline (PBS, Dulbecco's), pH 7.4,

adjusted to 10^8 cells ml^{-1} and incubated in PBS containing $10 \mu\text{M}$ CMP- ^3H -N-acetylneuraminic acid (CMP- ^3H -NANA) for 15 or 60 min at 37°C . It was assumed that under these conditions whole nucleotide monosaccharides do not enter the cell. When the cells were incubated with CMP- ^3H -NANA in the presence of a 100-fold higher concentration of free unlabelled N-acetylneuraminic acid, N-acetylmannosamine, mannosamine or mannose (all competitors of possible labelled degradation products of CMP- ^3H -NANA), there was no decrease in incorporation levels (Table 1, experiments 1 and 2). Apparently, extracellular degradation, enzymic¹⁰ or otherwise, of CMP- ^3H -NANA with subsequent uptake and incorporation of labelled products was not responsible for the cell labelling. In addition, paper chromatography⁸ of the cell medium following incubation with CMP- ^3H -NANA revealed that less than 1% of the total radioactivity was present as free ^3H -NANA. It was concluded, on the basis of the above, that incorporation of ^3H -NANA from CMP- ^3H -NANA into glycoconjugate must be taking place at the cell surface. We attempted to confirm this autoradiographically.

To attain sufficient incorporation levels of ^3H -NANA for EM autoradiography to be feasible, L-1210 cells were treated with *Vibrio cholerae* neuraminidase (VCN) for

15 min before incubation with CMP- ^3H -NANA. The procedure presumably increases the available endogenous acceptor sites at the external cell surface for ectosialyltransferase and provides a sixfold increase in incorporation of label without affecting cell viability (Table 1, experiments 1 and 2). After a 15-min incubation in $10 \mu\text{M}$ CMP- ^3H -NANA, cells were washed thoroughly in PBS, pelleted, fixed in 3% buffered glutaraldehyde (pH 7.0), postfixed in 1% osmium tetroxide, dehydrated and embedded in Spurr medium. Sections of the cells were processed for EM autoradiography^{11,12}. Only the monosaccharide incorporated into glycoprotein, and not that present in the cell as free sugar, nucleotide sugar or glycolipid, is retained after this tissue processing^{13,14}.

EM autoradiographs of L-1210 cells incubated with CMP- ^3H -NANA under the above conditions consistently showed grains over the plasma membrane of the cell (Fig. 1a). When for comparison, cells were labelled under the same conditions for 15 min with $10 \mu\text{M}$ ^3H -galactose (New England Nuclear, 0.6 Ci mmol^{-1}) in PBS and examined, the grains were concentrated mainly over the Golgi apparatus (Fig. 1b). A comparison of the relative grain densities over several cellular compartments (Table 2) clearly defines the organelles where incorporation of either label is taking place. The mode of incorporation of ^3H -NANA directly on to external plasma membrane glycoconjugate from its nucleotide monosaccharide represents a definite departure from that of free monosaccharides^{13,14} which are ordinarily incorporated by means

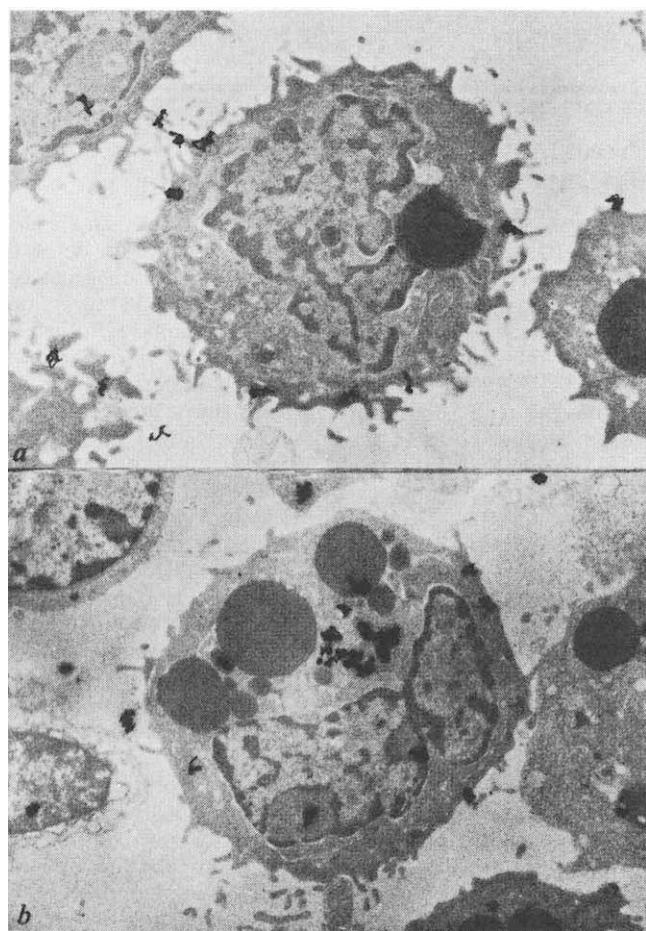


Fig. 1 Electron microscope autoradiographs of labelled L-1210 cells incubated 15 min with CMP- ^3H -NANA (a) or ^3H -galactose (b). The autoradiographic methods of Salpeter and Bachmann¹¹ were applied as described previously¹². Ultramicrotome sections ($1,000 \text{ \AA}$ thick) were mounted on collodionised slides, stained with 2% uranyl acetate, carbon coated and overlaid with a monolayer of Ilford L-4 emulsion. The preparations were exposed 25–55 d, developed in D-19 (Kodak) for 2 min at 24°C and photographed with a Siemens Elmiskop 101. Note that for cells labelled with CMP- ^3H -NANA, the grains lie mainly over the plasma membrane or within the range of radiation scatter from it while for cells labelled with ^3H -galactose, the grains are concentrated over the Golgi apparatus ($\times 2,772$).

Table 1 *In vitro* incorporation of N-acetylneuraminic acid from CMP- ^3H -NANA by murine leukaemic L-1210 cells

Experiment	Conditions	^3H -NANA incorporation (d.p.m. per 10^7 cells)	
		15-min incubation	60-min incubation
1	Cells	3,104	18,109
	Cells+NANA*	3,228	19,423
2	VCN-treated cells†	19,661	94,167
	VCN-treated cells+NANA	22,248	121,071
3	Cells labelled (21,100 d.p.m. per 10^7 cells) as in experiment 2 and post-treated 15 min with VCN‡	2,069	—
4	VCN-treated cells preincubated§		
	0 min in PBS	19,380	91,062
	30 min in PBS	18,800	92,101
	60 min in PBS	19,100	90,381

5×10^7 L-1210 cells were suspended in 0.5 ml PBS containing $10 \mu\text{M}$ CMP- ^3H -NANA (New England Nuclear, $2.33 \text{ Ci mmol}^{-1}$). The cell suspensions were incubated for 15 or 60 min at 37°C in a Dubnoff metabolic shaker. Viability remained $> 96\%$ throughout the experiments as measured by Trypan blue dye exclusion. Incubations were terminated by the addition of 2 ml of 1% phosphotungstic acid in 0.5 N HCl. Acid-insoluble material was collected by centrifugation (500g for 5 min) followed by two washes with 10% trichloroacetic acid. The insoluble material was washed once with ethanol:ether (2:1) and dissolved in 0.2 ml 1 N NaOH. This material was neutralised with 1 N HCl and the radioactivity determined by scintillation counting as previously described⁸. The incubations were performed in duplicate on at least three different occasions. The data are expressed as the means of those experiments.

*During the incubation in CMP- ^3H -NANA, 1 mM NANA was included in the medium as a competitor of possible CMP- ^3H -NANA degradation products. N-acetyl-mannosamine, mannosamine and mannose were also separately tested with results similar to those shown for NANA.

† 5×10^7 washed cells were treated with 10 units of *Vibrio cholerae* neuraminidase (Behring Diagnostic Company, EC 3.2.1.18) in 0.5 ml PBS for 15 min at 37°C . The cells were washed three times in 2 ml PBS before incubation with CMP- ^3H -NANA.

‡Cells labelled as in experiment 2 (—NANA, 15 min) were washed three times in 2 ml PBS, resuspended in 0.5 ml PBS containing 10 units of VCN and incubated for 15 min at 37°C . Acid-insoluble radioactivity was determined as above.

§ 5×10^7 washed VCN-treated cells were suspended in PBS and preincubated for 0, 30 or 60 min at 37°C . CMP- ^3H -NANA was added to the same medium to a final concentration of $10 \mu\text{M}$ and the cells were incubated for 15 or 60 min at 37°C .

of multiple activation steps in the Golgi apparatus as exemplified here with galactose, a known¹³ glycoconjugate marker.

To demonstrate that the radioactivity associated with the cells was actually ³H-NANA, labelled cells were exposed to VCN for 15 min. The enzyme released 90% of the bound radioactivity from the cells (Table 1, experiment 3). Paper chromatography⁸ revealed that 86% of the radioactivity recovered into the medium was ³H-NANA and 14% CMP-³H-NANA. This latter may be a result of incomplete washings after the labelling period.

It is conceivable that the transfer of ³H-NANA on to the cell surface might have been catalysed by free enzyme in the incubation medium incidentally secreted by Golgi vesicles¹⁵ or otherwise released into the medium by cell surface shedding. To test this possibility, washed VCN-treated cells were preincubated 0, 30 or 60 min in PBS before the addition of CMP-³H-NANA to the cell medium, to allow enzyme accumulating in the cell medium during the preincubation period to then catalyse higher levels of incorporation in the 30 or 60-min preincubation samples. But in fact, the 30 and 60-min preincubation samples resulted in no additional incorporation during subsequent incubation in the presence of CMP-³H-NANA (Table 1, experiment 4). This indicates that labelling of the cell surface is not a result of a sialyl-transferase being released into the medium from the cells before or during the labelling period. Further, there was no detectable transfer of ³H-NANA on to cells treated at 56 °C for 60 min (to inactivate endogenous cell surface enzymes) by the medium from a 15-min preincubation with viable cells.

The ultrastructural data reported here, together with those obtained biochemically, are evidence for the presence of an ectosialyltransferase system at the surface of L-1210 cells. We note that the bound radiation demonstrated at this location does not represent the enzyme itself, but macromolecular acceptors that have been glycosylated by the sialyltransferase. As with most EM cytochemical techniques for localising enzymes (with exceptions such as the labelled-inhibitor methodology¹⁶), it is the product of the enzyme reaction that is demonstrated and not the enzyme itself. The precise role of such enzymes or their acceptors is uncertain. Roseman² has postulated that batteries of different glycosyltransferases on the plasma membrane bind to acceptor molecules on opposing cell surfaces in a lock and key fashion to establish cell recognition and/or early adhesion. This postulate has recently gained much experimental support from several biochemical studies⁴⁻⁷. A more direct assessment of the role of ectoglycosyltransferases (or the macromolecules they glycosylate) in cell behaviour may now be achieved through the application of the methodology used in this study. In addition, the enzyme(s) may

serve as a selective means for physiologically introducing an isotopic label on to specific cell surface glycoconjugates.

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Serological identification of HL-A-linked human 'Ia-type' antigens

THE Ir genetic region of the mouse which lies between the H-2K and H-2D loci that control the original serologically detectable H-2 specificities, has also been shown to contain genes controlling a new set of serological specificities called Ia for immune associated. Antisera raised by cross immunisation between H-2 recombinant strains have been shown to recognise the products of several genes within the Ir region (reviewed in ref. 1). Unlike the H-2K and H-2D antigens the Ia antigens have a tissue distribution that often does not include T lymphocytes.

By analogy with the mouse, human Ia antigens have been defined by mixed lymphocyte culture (MLC) inhibition and by serological tests on HL-A identical unrelated individuals²⁻⁵. We describe here some new approaches to the ready identification of sera against Ia-type antigens in man which make use of lymphoblastoid cell lines derived from B cells⁶.

Extra reactions of HL-A-typing sera with lymphoid lines have been described^{7,8}. A similar increase in reactivity has been noted when typing chronic lymphocytic leukaemia (CLL) cells derived from B cells⁹⁻¹¹. Using HL-A-negative variants of lymphoid lines some of these extra reactions have been shown to be caused by antigens other than HL-A¹².

The fact that all these anomalous reactions are with cells presumed to be derived from B lymphocytes suggested that they may be directed against polymorphic antigens specific to B lymphocytes. Furthermore, the suggestion¹² that antibodies to these antigens tended to occur in HL-A-typing sera containing antibodies to particular serologically-defined LA and four locus antigens, indicated an association between the production of these antibodies and particular HL-A antibodies. This could most easily be explained by linkage disequilibrium¹³ between the genes determining the corresponding antigens which, together with the evidence for B cell specificity, suggested that the anomalous reactions with lymphoid lines and CLL cells may be directed against human equivalents of the mouse H-2-linked Ia antigens.

In the first systematic screen for such reactions we used the B-cell-derived lymphoid lines T51 (ref. 14) and DAUDI (ref. 15), and our standard fluorochromatic cytotoxicity assay. DAUDI does not express the usual serologically determined

Table 2 Grain density distributions for cells incubated with CMP-³H-NANA or ³H-galactose

Compartment	Relative grain density	
	CMP- ³ H-NANA	³ H-galactose
Plasma membrane*	100	16
Cytoplasm	11	14
Osmiophilic vacuoles	0	14
Golgi apparatus	7	100
Nucleus	2	7
Nucleolus	0	5

Photographs of labelled cells were taken at random and enlarged to a final magnification of $\times 10,000$. Grains and points from a superimposed grid (1 point = $9 \mu\text{m}^2$ at $\times 10,000$) were scored according to the cellular compartment they occupied. Grains per μm^2 minus background (0.2 grains per $100 \mu\text{m}^2$) were determined for each compartment and then normalised so that the compartment with the highest grain density was set equal to 100 and all other densities for that label adjusted proportionally. Relative grain densities for cells labelled with CMP-³H-NANA are based on 230 cells, 780 grains and 1,300 grid points. Those for ³H-galactose are based on 175 cells, 850 grains and 1,200 grid points.

*Includes all grains and points within 3,000 Å of the actual membrane to allow for radiation scatter¹².

Table 1 Reactions of the sera P1530B and P2604B with peripheral blood lymphocytes of normal donors

Donor*	P1530B % +ve cells	P2604B % +ve cells
HB	24%	21%
EB	26%	20%
DB	14%	ND
EP	20%	ND
JB	<1% [†]	ND
WB	20%	22%
JGB	26%	27%
LJ	25%	26%
JG	2%	ND
GP†	<2% [‡]	ND
JJ	29%	ND

*Lymphocytes separated from defibrinated blood on a Ficoll-Trisil gradient and stored frozen in liquid nitrogen.

†Cells of serum donor confirming the polymorphism.

‡Results giving <5% positive cells were considered to be negative for a given serum.

ND, Not determined.

HL-A antigens of the LA and four loci, nor does it have β_2 microglobulin (β_2m)¹⁶. Any sera reacting with DAUDI are therefore presumed to have antibodies against Ia-type antigens. Out of 108 pregnancy sera previously characterised as having lymphocytotoxic HL-A antibodies when screened on a cell panel covering all the known LA and four loci antigens, ten were found which reacted with DAUDI or gave reactions with T51 that could not be explained by its HL-A type.

Sera P1530B, a weakly cytotoxic anti-HL-A9 antibody, was selected for further investigation using an indirect immunofluorescence assay¹⁶. Table 1 suggests that P1530B was reacting preferentially with B cells of some, but not all donors. As further evidence for B cell specificity, the percentage of lymphocytes reacting with P1530B was compared with the percentage reacting with a fluorescent anti-human IgM antibody. The differences were not significant in five cases tested. Furthermore, labelling with anti-human IgM antibody and P1530B failed to increase the percentage of fluorescing cells. B lymphocytes separated from peripheral lymphocytes by 'E' sheep red cell rosetting also showed an increased percentage of positive cells with P1530B, the rosetted T cells being totally unreactive. The positive reactions could not be removed by absorption with HL-A9-positive platelets (4×10^9 ml⁻¹) which did remove the weak cytotoxic HL-A9 antibody.

Two lines of evidence indicate that the P1530B reaction with B lymphocytes is directed against an antigen determined by a gene in the HL-A region, thus supporting the analogy with the mouse Ia antigens. The first is that out of a series of six somatic cell hybrids derived from a cross between the mouse L cell derivative A9 and DAUDI (refs 16 and 17) the only positive clone by immunofluorescence was also the only one containing human chromosome 6, which carries the HL-A region^{18,19}. Subclones of this and other similar hybrids are now being studied to confirm this assignment. The second is that the results of a study of the immunofluorescence reactions of

P1530B on peripheral blood lymphocytes of parents and offspring from three families, were consistent with linkage between the HL-A system and the gene determining the antigen recognised by P1530B.

Screening for serum reactions similar to P1530B was extended by testing a panel of HL-A2 antisera for their cytotoxic activity on DAUDI, 8866 and its HL-A2-negative variant 8866 1-2 (ref. 12). Reactions with 8866 1-2 or DAUDI, are indicative of Ia-type reactivity. Out of 13 such sera tested, all of which reacted with 8866, two (5005 and 7155A, Table 2) also reacted with 8866 1-2 and DAUDI, whereas the remaining 11 were negative on these lines. This approach to detecting Ia-type activities, however, is limited by the need for a number of well-characterised HL-A-negative lymphoid line variants. It did

Table 2 Screen of HL-A2 antisera on Bri8 before and after stripping with anti- β_2m and horse anti-rabbit IgG in standard cytotoxic assay

Sera	Origin	BR18	BR18 stripped
4025C (Pinquette)	NIH Walford	4	1
7086A (EYR/8573)	Sheffield	3	1
4245A (7234)	van Rood	3	1/2
4253A (Butterfield)	Rodey	3	4
1087B (Di Maggio)	Bodmer/Payne	2	1
P3098B	Bodmer (Oxford)	4	1
P3229B	Bodmer (Oxford)	4	1
7155 (CIC111544)	Sheffield	3	4
5005 (CLB11)	Engelfriet	4	4
5221 (Jochum)	E. Cohen	4	1/2
3002X (Caminiti)	Bodmer/Payne	4	1
Rabbit anti- β_2m	Dakopatts	4	1/2

Scores: 4, very strong positive; 3, strong positive; 2, weak, but definitely positive; 1/2, slight reaction, doubtful positive; 1, negative. Stripping of β_2m was carried out essentially according to Bernoco *et al.*¹⁹ using rabbit anti-human β_2m (Dakopatts, Denmark) followed by a purified horse anti-rabbit IgG antibody, which was absorbed with an equal volume of human red blood cells and 10^8 DAUDI cells per ml serum to remove nonspecific effects. Stripped cells were suspended in a medium containing 10 mM sodium azide and then used in a cytotoxicity assay.

however, suggest another way of screening sera for Ia-type activity, namely to remove HL-A from lymphoid lines or from peripheral blood lymphocytes by 'stripping'¹⁹ with anti- β_2m and anti-immunoglobulin sera.

The removal of β_2m from the surface of peripheral blood lymphocytes in this way also removes the associated allogeneic chain of the HL-A molecule and thus results in a complete loss of HL-A antigenicity. As DAUDI seems to have Ia-type antigens on its cell surface but not β_2m , at least some of these Ia antigens are presumably not normally associated with β_2m on the cell surface and so would not be removed from normal cells by stripping of the β_2m with specific antisera. In this way, therefore, we can turn any line effectively into a completely HL-A-negative variant and then screen for other Ia-type activities not associated with β_2m .

Reactions of the line Bri8 (HL-A1, 2, 8, 13) before and after

Table 3 Reaction of lymphoid lines with selected pregnancy sera after stripping with anti- β_2m

Cells	HL-A type	Sera									
		P1048B	P1530B**	P2242B	P2318B	P2377B	P2482B	P2604B	P2762B	P2902B	P2961B
Bri8*	1, 2, 8, 13	1	2	3	4	2	1	3	1	3	4
T51†	1, 2, 8, 27	4	4	4	4	3	4	1	1	3	3
8866‡	2, 3, 7—	1	4	1	4	3	3	4	1	4	1
MICH§	2, 32, 15, 17	1	2	1	1	1	4	4	4	1	4
BR17	2, 9, 12, 17	3	1	4	3	1	1	4	1	1	4
DAUDI¶	—	2	4	2	3	4	3	2	4	4	4

Sera (including P1530B) were selected in the course of routine screening of pregnancy sera for HL-A activity because of their reactions with DAUDI. Scoring system as in Table 1.

*Cells from Searle Diagnostics; †see text; ‡cell line initiated by Dr G. Moore; §cells from Searle Diagnostics; ||cells from Searle Diagnostics; ¶see text; **P1530B was also found to be positive on the lymphoid lines Ba-13, Des3 and Sha, and negative on the lymphoid lines OR-1 and Bec 11.

stripping with essentially the same set of HL-A2 antisera used on 8866 and its variant, are shown in Table 2. The reactions of HL-A2-positive peripheral lymphocytes with this set of antisera could be completely removed by β_2m stripping. The same two sera (5005 and 7155) that reacted with the HL-A-negative variant of 8866 (and with DAUDI) also reacted with β_2m stripped Bri8. The only other serum to react in this way was 4253A, all the remaining sera, including as a control the anti- β_2m , were negative. These results validate the use of stripped lymphoid line cells for the detection of Ia-type activity. Reaction of 4253A with stripped Bri8 but not 8866 1-2 or DAUDI indicates some complexity in the Ia polymorphism. This is confirmed by more extensive typing of several of the lymphoid lines after β_2m stripping with a total of 20 antisera that have been shown to exhibit these Ia-type activities. A sample of these reactions is shown in Table 3. There is a complex pattern of reactions which is reminiscent of the early days of HL-A typing with unselected pregnancy sera, before the serologically detectable antigens had been properly defined. It seems likely that these initial Ia antisera will be complex mixtures of possibly cross reacting antibodies and further work is now being carried out to define the specificities properly.

Very striking associations between HL-A antigens and specific diseases have been established^{20,21}. There are, however, a number of weak associations with the serologically detectable antigens which may simply reflect weak linkage disequilibrium between the genes in the HL-A region controlling disease susceptibility and those controlling the antigens of LA, 4 and AJ series. MLC typing in multiple sclerosis patients²² has demonstrated that in such cases alleles at other loci in the region may show much stronger associations and so lead to both a better definition of the HL-A association and possibly its cause. Serological Ia typing will undoubtedly prove to be a powerful tool in future disease association studies.

While this paper was being written, Winchester *et al.*²³ reported a somewhat analogous study on B lymphocyte specific human antigens also using lymphoid lines. Details of our investigations will be published elsewhere.

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Mouse T-cell surface glycoprotein recognised by heterologous anti-thymocyte sera and its relationship to Thy-1 antigen

CELL surface proteins specific for either T or B lymphocytes probably have a significant role in lymphocyte physiology, and we have therefore been characterising the plasma membrane proteins from mouse lymphocytes to identify such molecules and to determine their function. We have previously detected a high molecular weight T-cell-surface protein (T200, apparent molecular weight 200,000) which could be labelled by lactoperoxidase-catalysed iodination¹ and was recognised by rabbit anti-mouse thymocyte sera (ATS). We have now identified a low molecular weight T-cell-specific molecule present on mouse thymocytes, T lymphomas and peripheral T cells which is also recognised by rabbit antibodies to mouse lymphocytes.

This molecule was recognised during immunoprecipitation studies using labelled thymocytes and both unabsorbed ATS and a preparation of ATS made specific for T cells by absorption. ATS selectively precipitated two major iodinated species from labelled thymocytes—T200 and a low molecular weight component of broad electrophoretic mobility (T25, molecular weight 25,000-30,000; Fig. 1b). Several less heavily labelled species were also precipitated by ATS. Qualitatively similar results were obtained with four different preparations of antisera: two raised as described previously², one by repeated intravenous immunisation³, and the fourth was a commercial preparation of antiserum (Microbiological Associates).

Similar gel profiles were observed whether cell lysates were prepared and immunoprecipitations carried out in the presence of either Nonidet P40 or sodium deoxycholate. Rabbit antisera to mouse T lymphomas that express the Thy-1 antigen also precipitate T25 and T200 (for example, anti-BW5147 serum, Fig. 1e, and anti-WEH1 22 serum, Fig. 1f). A preparation of ATS which was absorbed with neonatal liver cells and spleen cells from thymectomised, irradiated, neonatal liver cell-restored mice and was specific for mouse T lymphocytes as judged by indirect fluorescence and cytotoxicity studies (anti-T serum^{4,5}), still contained antibodies which recognised T25 (Fig. 1c). This observation indicates that T25 carries antigenic determinants which are specific for T lymphocytes. The amount of radioactivity associated with T25 suggests that it is a major component of the thymocyte cell surface. Almost all the radioactivity precipitated by the anti-T cell serum was associated with T25 and this antiserum precipitated 4.8% of the total acid-insoluble radioactivity in an NP40 extract of labelled thymocytes. In contrast, unabsorbed ATS precipitated 8.5% and normal rabbit serum (NRS) 0.16% of the acid-insoluble radioactivity. T25 is also present on the surface of T lymphomas which express the Thy-1 antigen (for example, WEH1 22, Fig. 1g).

Examination of the labelled species precipitated by unabsorbed ATS or anti-T serum from iodinated peripheral lymphocytes of either normal or congenitally athymic (*nu/nu*) mice showed that T25 is present on peripheral T cells but probably in lesser amounts than on thymocytes (Fig. 2). Anti-T serum precipitated T25 from detergent-solubilised extracts of spleen and lymph node cells of normal mice (Fig. 2c and e) but not from peripheral lymphocytes of athymic mice. Unabsorbed ATS precipitated T25 from spleen and lymph node cells of normal mice, and T200 and the high molecular weight B-cell protein¹ from peripheral lymphocytes of both normal and athymic mice (Fig. 2b, d and e). A comparison of the gel profile of the labelled species precipitated by ATS with that of the labelled proteins precipitated by rabbit anti-mouse immunoglobulin serum (Fig. 2h) suggests that this particular preparation of ATS reacts with immunoglobulin.

Thus, T25 is a T-cell-specific molecule present on thymocytes, T lymphomas and peripheral T cells. Biosynthetic labelling studies, sensitivity to proteolytic enzymes and binding to plant

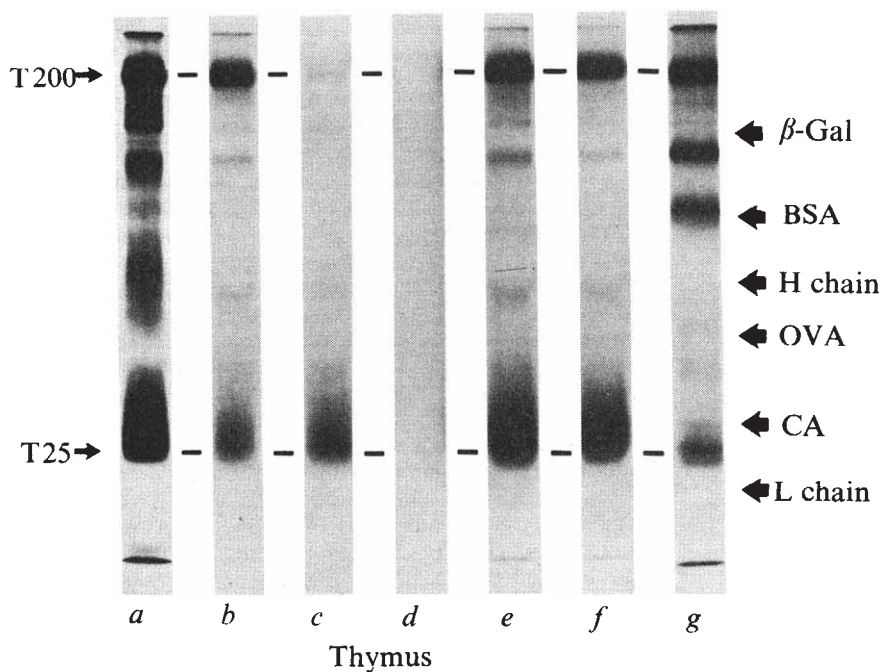


Fig. 1 Autoradiographs of iodinated proteins of BALB/c thymocytes. *a*, Total thymocyte NP40 lysate. *b-f*, Immunoprecipitates of thymocyte NP40 lysate using various antisera: *b*, unabsorbed ATS; *c*, anti-T serum; *d*, NRS; *e*, anti-T lymphoma BW5147; *f*, anti-T lymphoma WEH1 22; *g*, immunoprecipitate of an NP40 cell lysate from WEH1 22 prepared with anti-WEH1 22 serum. Molecular weight standards: β -Gal, β -galactosidase; BSA, bovine serum albumin; H chain, rabbit IgG heavy chain; OVA, ovalbumin; CA, carbonic anhydrase; L chain, rabbit Ig light chain. A 9% polyacrylamide gel was used. The preparation of viable cell suspensions, lactoperoxidase-catalysed iodination, solubilisation of cells for immunological studies, and polyacrylamide gel electrophoresis in the presence of SDS have been described¹.

lectins suggest that T25 is a glycoprotein (I.S.T. and M.J.B., unpublished). Furthermore, no radioactivity was precipitated from an NP40 lysate of cells labelled with ³H-palmitate using a variety of antisera which precipitate T25 and T200, suggesting neither molecule is associated with lipid in these conditions. Other investigations to be reported in detail elsewhere suggest that T25 is identical with the molecule which carries the mouse Thy-1 alloantigenic determinant⁶. T25 is precipitated by rabbit antisera against mouse brain, rat thymocytes and rat brain; similar cross reactivity has been noted for the Thy-1 molecule on the surface of mouse⁷ or rat thymocytes⁸. T25 cannot be detected on the surface of Thy-1-negative variants⁹ of mouse T lymphoma cell lines, and has the same properties as those of the rat thymocyte Thy-1 molecule¹⁰ when chromatographed on Sepharose 6B in 1% sodium deoxycholate. T25 is recognised by a rabbit antiserum raised against a purified preparation of rat brain Thy-1.1 antigen (Fig. 3), also indicating that T25 carries the Thy-1 determinant. This antiserum is specific for the Thy-1 molecule on rat thymocytes (A. N. Barclay, M. Letarte-Muirhead, and A. F. Williams, unpublished). Antibodies present in this antiserum which cross react with T25 from BALB/c thymocytes were removed by absorption with either mouse thymocytes, mouse brain homogenate, or rat thymocytes,

but not by rat lymph node cells which express only low levels of the Thy-1 antigen^{11,12}. In contrast to this heterologous antiserum, mouse anti-Thy-1 alloantisera failed to specifically precipitate any iodinated species from thymocytes or T lymphoma cells whether solubilised in NP40 or sodium deoxycholate. This failure is probably a consequence of the low affinity of the anti-Thy-1 alloantibodies present in these sera. The presence of detergent or the abolition of multivalent interactions by disruption of the cell membrane may prevent formation of stable antibody-antigen complexes.

Two other possible explanations, that detergent specifically masks the Thy-1 antigenic determinant, or that the mouse Thy-1 antigenic determinant is a glycolipid^{13,14} which is specifically associated with T25 on the T lymphocyte surface but which dissociates from T25 after solubilisation of the cell membrane by detergent can be excluded because an antiserum prepared from the rabbit anti-rat Thy-1 serum by absorption with BALB/c thymocytes or brain tissue precipitated T25 from an NP40 lysate of AKR/J thymocytes but not BALB/c thymocytes. The recognition of T25 by an antiserum apparently specific for the Thy-1.1 alloantigenic determinant strongly suggests that T25 is identical with the Thy-1 antigen; only the unlikely possibility that BALB/c and AKR/J mice express

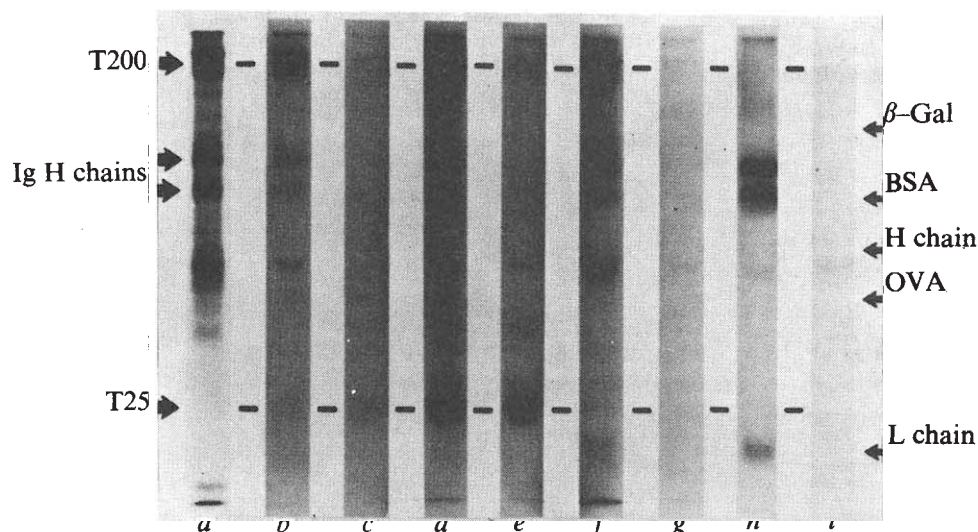


Fig. 2 Autoradiographs of iodinated peripheral lymphocytes from normal and congenitally athymic (*nu/nu*) mice: *a-c*, spleen cells; *d* and *e*, lymph node cells from BALB/c mice; *f-i*, mixture of spleen and lymph node cells of mice carrying *nu/nu* character backcrossed on to a BALB/c genetic background. *a*, Total spleen cell NP40 lysate; *b-i*, immunoprecipitates using various antisera; *b*, *d* and *f*, unabsorbed ATS; *c*, *e* and *g*, anti-T serum; *h*, anti-mouse Ig serum; *i*, NRS. A 9% polyacrylamide gel was used.

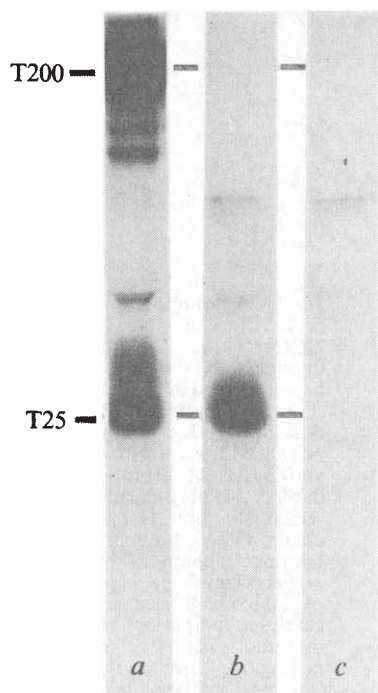


Fig. 3 Precipitation of T25 by rabbit anti-rat Thy-1 serum. Immunoprecipitates prepared from NP40 lysate of BALB/c thymocytes with: *a*, ATS; *b*, anti-rat Thy-1; *c*, NRS. A 7.5–15% linear polyacrylamide gradient gel was used. Note that two iodinated species from thymocytes which are precipitated by ATS but not by anti-rat Thy-1 serum, are resolved from T25 on the 7.5–15% polyacrylamide gradient gel. These species are not resolved from T25 on a 9% polyacrylamide gel and this, in part accounts for the apparent heterogeneity of T25 from thymocytes seen in Figs 1 and 2.

different alleles of another alloantigenic determinant with the same tissue distribution and interspecies cross reactivity as Thy-1, prevents these observations from being considered formal proof that T25 is the Thy-1 antigen. If this is the case then earlier reports that the mouse Thy-1 antigen is a glycolipid^{13,14} require re-examination. One possibility is that the Thy-1 antigenic determinant may be carbohydrate; the blood group substances provide an example of a carbohydrate antigen present on both glycoprotein and glycolipid¹⁵.

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Thymus dependence of viral antigens

AFTER exposure of animals to most antigens, formation of antibody by cells of the B lymphocyte lineage requires helper effects of T lymphocytes¹. The degree of thymus dependence varies for different antigens, and slowly metabolised immunogens with repeating epitopes, generally polysaccharides or polymerised forms of protein, seem to be the most thymus-independent². Antigens of some viruses, however, may be relatively thymus independent. Inherent in the structures of many viruses is a rigid geometrical array of repeating antigenic determinants which, if the spatial relationships were appropriate, may effectively interact with and trigger B cells without help from T cells. Also, glycoproteins are important antigens in the virions of many viruses³, and the carbohydrate portions of these molecules may function as thymus-independent immunogens. In addition, repeated immunisation of mice depleted of T cells can sometimes result in antibody production to thymus-dependent antigens⁴, and virus infections often result in the sustained production of antigens and thus prolonged exposure of the infected host to the antigens. These antigens may be presented to the immune system in a variety of ways—on the membranes of infected cells, as soluble antigens released from such cells, and as repeating epitopes on virus particles.

The humoral antibody responses of athymic nude (*nu/nu*) mice after virus infection were compared with those of normal littermates (*nu/+* or *+/+*). Lymphocytes from nude mice have no demonstrable functions characteristic of T cells⁵, whereas B cell functions such as blastogenic responses to lypopolysaccharide are present, and normal or increased antibody responses are made to thymus-dependent antigens such as type III pneumococcal polysaccharide⁶. The function of dendritic macrophages in nude mice is also normal⁷ and nude mice can be rendered immunologically competent by the implantation of thymus grafts⁸. Heterozygous (*nu/+*) mice seem to be immunologically normal⁵. The alternative use of mice which have been depleted of T cells by thymectomy, irradiation, and bone-marrow reconstitution (TXBM) was rejected as such mice have defects in addition to T-cell depletion of T cells^{9–11} (W. H. B. and H. C. Morse, unpublished).

Nude mice and their normal littermates were infected intraperitoneally and their sera assayed for antibody to certain antigens (Table 1). Sustained antibody responses to all 12 viruses from nine major virus groups were markedly thymus dependent. Nude mice, however, made early transient responses equal in magnitude to those of normal littermates to the picornaviruses, Coxsackie B1 virus and encephalomyocarditis (EMC) virus, to vesicular stomatitis virus (VSV), and to Sindbis virus. Antibody production in nude mice to the Sindbis antigen(s) that react with neutralising antibody was sustained for 2 weeks and was solely IgM as indicated by its appearance in the IgM fraction after column chromatography of serum on Sephadex G-200 and by its sensitivity to 2-mercaptoethanol (data not shown). Normal littermate mice produced IgG as well as IgM antibody to this antigen and these mice, unlike nude mice, produced large secondary responses (1:10,000) of predominantly IgG antibody. The Sindbis antigen(s) concerned are found on two virion membrane glycoproteins^{12,13}.

As Kennedy demonstrated that the antigenic determinants of Semliki Forest virus (an alphavirus similar to Sindbis virus) are in the polypeptide portions rather than the carbohydrate portions of the envelope glycoproteins¹⁴, it will be of interest to determine if antibodies produced in the relatively thymus-independent IgM response react with antigenic determinants of Sindbis virus residing in the protein part of the molecule. Neutralising-antibody production by nude mice to Coxsackie B1 virus, EMC virus, and VSV peaked 6–8 d after infection, was sensitive to 2-mercaptoethanol, and thus was probably IgM immunoglobulin. As picornaviruses back lipid and carbohydrates, the neutralising antibody to Coxsackie B1

Table 1 Antibody titres in nude mice and littermate controls after virus infection

Genus	Virus	Assay*	Inoculum†	Immunisation schedule‡	Assay day§	Antibody titre	
						Nude	Littermate
Picornavirus	Coxsackie B1	NT	10 ⁶ PFU	0	6	512	512
			10 ⁵ PFU	0	10	16	1,024
	Encephalomyocarditis	NT	10 ³ PFU	0	7	256	256
			10 ³ PFU	0	10	16	1,024
Orthomyxovirus	Influenza, PR8	HI	10 ³ PFU	0+10	17	<8	2,048
			500 HAU	0	10	<2	256
			500 HAU	0	12	<2	256
			500 HAU	0	18	<2	256
Paramyxovirus	Simian virus 5	NT	10 ³ PFU	0	7	<8	32
			10 ³ PFU	0	10	<8	128
	Sendai	HI	100 HAU	0	13	<10	80
			100 HAU	0+14	21	<10	640
Rhabdovirus	Mumps	NT	10 ⁴ PFU	0+14+21	28	<10	1,280
			10 ⁴ PFU	0+13+26	33	<8	64
	Vesicular stomatitis	NT	10 ⁶ PFU	0	7	256	512
			10 ⁶ PFU	0	10	8	2,048
Togavirus	Sindbis, Egyptian 101	NT	10 ⁶ PFU	0+10	17	16	>2,048
			10 ⁵ PFU	0	2	32	32
			10 ⁵ PFU	0	4	128	128
			10 ⁵ PFU	0	6	256	256
Parvovirus	Minute virus of mice	HI	10 ⁵ PFU	0	10	1,000	1,000
			10 ⁵ PFU	0	7	<20	20
			10 ⁵ PFU	0	9	<20	40
			10 ⁵ PFU	0	10	<20	80
Adenovirus	Mouse adenovirus	CF	10 ⁵ PFU	0+12	17	40	160
			10 ⁵ PFU	0+12+21	26	20	160
			10 ³ PFU	0	7	<10	<10
			10 ³ PFU	0	11	<10	20
Herpesvirus	Herpes simplex, type 1	NT	10 ³ PFU	0	15	<10	>80
			10 ⁵ PFU	0	6	<8	64
Papovavirus	Simian virus 40	NT	10 ⁵ PFU	0	14	<8	128
			10 ⁷ PFU	0+5+17	27	<4	128

*NT, neutralising antibody; HI, haemagglutination-inhibiting antibody; CF, complement-fixing antibody.

†Immunising dose of infectious virus given intraperitoneally on day 0 and repeated thereafter as indicated in immunisation schedule.

‡Mice were inoculated on day 0 and on subsequent days as indicated.

§Antibody assays were carried out on indicated day after initial inoculation. Assays were carried out soon after infection to detect transient IgM responses, as well as late in infection and after multiple immunisations. Neutralisation tests were carried out by incubating 0.2 ml virus containing 100 plaque-forming units (PFU) and 0.2 ml diluted sera at 37 °C for 1 h and assaying for residual infectious virus. Complement-fixation (CF) and haemagglutination-inhibiting (HI) tests were carried out on coded serum samples by Microbiological Associates, Bethesda, Maryland. All sera were heated to 56 °C for 30 min before antibody assay. Titres are expressed as the reciprocal of the highest serum dilution that neutralised 50% of the virus and are the average of determinations on two or more animals.

virus and EMC virus must be directed against protein antigenic determinants. The neutralisation antigen(s) of VSV are found on an envelope glycoprotein, but the relative contributions of protein and carbohydrate to the antigenic determinants are not known.

Until availability of the athymic nude mouse, however, neonatal thymectomy or treatment with ALS was used to deplete animals of T lymphocytes and ascertain the thymus independence of viral antigens. Thus, neither neonatal thymectomy⁴ nor ALS treatment¹⁵ of mice diminished their production of HI antibody to influenza virus. In CF1 mice neonatally thymectomised, titres of complement-dependent neutralising antibody (probably IgM) to herpes simplex virus (HSV) were similar to those in control mice¹⁶. Discrepancies between these findings and the present demonstration of the thymus dependence of antibody production to influenza virus and HSV may stem from the use of mice inadequately depleted of T cells in earlier studies. Thymus dependence of HI antibody production to influenza virus has, however, been observed using TXBM¹⁷ and nude¹⁸ mice; the former do not produce neutralising antibody to HSV (W. H. B. and H. C. Morse, unpublished). Also, TXBM mice have been reported to respond well to Coxsackie B3 virus¹⁹.

In conclusion, normal magnitude but transient IgM responses have been observed early after infection of athymic nude mice with several viruses. The antigens concerned are clearly protein in nature (picornaviruses) or are probably protein portions of virion envelope glycoproteins (Sindbis virus and VSV). The switch from IgM to IgG synthesis and the elicitation of secondary (IgG) antibody responses to these viral proteins, as to protein antigens generally, is highly thymus-dependent.

The present study has, however, surveyed the thymus dependence of a small number of viral antigens and utilised mice

exclusively; the examination of other antigens of these same viruses, of different viruses, or the use of other animal species may disclose more thymus-independent viral antigens. Also, because of a limited supply of nude mice, dose-response studies were not carried out. Although normal littermates (*nu*/+ or +/+) were used in this study for comparisons, a more proper control would be thymus-grafted nude mice using thymuses from congenic mice. As the nude mice available were the result of passing the *nu* trait in outbred Swiss mice, such experiments were impossible and must await the availability of nude mice congenic with an inbred strain. The use of infectious agents makes difficult the administration of precise amounts of antigens, and the possibility exists that the viral infection itself may be thymus-dependent. Antigenic doses, however, were in some cases sufficient to elicit normal IgM responses in nude mice and the failure to switch to sustained IgG responses is unlikely to be caused by insufficient antigen.

Our findings should be considered in the light of the need for cell-mediated immunity in viral infections. Depletion of T cells may result in deficient antibody production as well as a diminution of effector cells involved in cell-mediated immunity. K-cell activity may also be diminished in those situations in which IgG antibody is not produced²⁰.

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Binding of α -foetoprotein to murine T cells

α -FOETOPROTEIN (AFP), an embryo-specific glycoprotein, is primarily known as a tumour-associated embryonic antigen. Although much is known about the occurrence of AFP in ontogeny and levels of AFP in different diseases, such as hepatocellular carcinoma and other tumours¹⁻⁴, little work has been done on its functional significance. Results from our laboratory have shown that AFP exerts an immunosuppressive effect on antibody synthesis when administered *in vivo*⁵ and that it is a non-cytotoxic suppressor of both the primary and secondary antibody response to sheep red blood cells (SRBC) *in vitro*⁶. In addition, we found that AFP suppresses certain T-cell-dependent functions in mice such as allogeneic and mitogen-induced lymphocyte transformation⁷. A recent report⁸ suggests that bovine foetuin is immunosuppressive for mitogen and allogeneic reactions. Bovine foetuin and AFP have, however, been shown to be different proteins⁹. Moreover, in view of the relatively large amounts of foetuin used to obtain suppression (10,000-fold higher than those required for suppression in our studies using AFP), it is possible that the foetuin preparations were contaminated with suppressive amounts of bovine AFP.

To define the cell type(s) affected by AFP we studied the binding of AFP to the surface of various cells involved in the immune reaction using direct immunofluorescence on preparations of thymus derived lymphocytes (T cells), bone marrow derived lymphocytes (B cells) and macrophages.

Mouse amniotic fluid (MAF) contains three major components: AFP, albumin, and transferrin⁶. MAF was collected from Ha/ICR mice in the late second trimester of pregnancy. Antiserum to MAF was prepared in rabbits by subcutaneous injection of MAF in Freund's complete adjuvant. A globulin fraction of this antiserum (double 18% sodium sulphate precipitation) was conjugated with fluorescein and absorbed with lyophilised normal adult mouse serum, rendering it monospecific for AFP. This antiserum gave a single line on Ouchterlony gel diffusion with MAF, which fused in a reaction of immunological identity with foetal, newborn and pregnant sera and purified AFP. Purified AFP was isolated from MAF as described previously⁶. Briefly, this involves affinity chromatography using an anti-whole adult mouse serum, followed by preparative polyacrylamide gel electrophoresis. The specificity of the AFP was determined by gel diffusion and immunoelectrophoresis using anti-mouse whole serum and anti-mouse MAF. The antisera were shown to be specific by the following: First, the absorbed antiserum gave a single line with whole concentrated MAF. This line fused on Ouchterlony with a single line in cord serum, pregnant serum and a highly purified preparation of AFP. Second, the antiserum was checked against another antiserum from Roswell Park, which is specific for AFP derived from a mouse hepatoma, and was found to form a line of

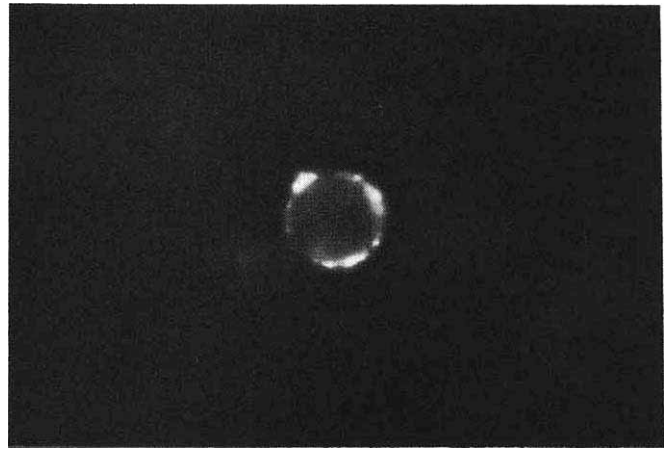


Fig. 1 A photomicrograph of C57BL mouse spleen cells incubated with MAF for 90 min, then stained with anti-AFP conjugated with fluorescein isothiocyanate.

identity. Third, the fluorescent reaction was blocked by pure AFP and not albumin or transferrin (see ref. 6 for techniques of isolation and purity of preparations and antisera). In addition to the procedures outlined in that reference, we used gel filtration on Sephadex G-200. Finally, at least three other antisera have been produced in this laboratory recently against purified preparations of AFP and have been shown to be identical to the one used in the present study. No significant surface staining occurred with an anti-mouse albumin. (In addition the purity of the AFP was checked by sedimentation ultracentrifugation and polyacrylamide gel electrophoresis in both alkaline and sodium dodecyl sulphate gels.)

All studies were carried out using 6-10-week-old C57BL female mice with the exception of the experiment done with nude mice. We found that after a 90-min incubation in MAF a maximum number of cells stained in whole spleen preparation (approximately 18%) as shown by positive membrane fluorescence when reacted with anti-AFP. Various primary and secondary lymphoid tissues were examined to determine the presence of cells which bound AFP. The results presented in Table 1 show that spleen, lymph node and cortisone resistant thymocytes, but not bone marrow or nude mouse spleen cells, contained AFP binding cells.

Adult mouse spleen cells were treated with either anti-Fab or anti- θ plus complement to produce lymphoid preparations depleted of B and T cells respectively¹⁰. Spleen cells were passed sequentially over glass and nylon wool columns to enrich for T cells¹¹. These preparations contained 88% cells staining with anti- θ and 7% cells staining with anti-Fab (anti-L chain). Peritoneal exudate cells were obtained by injection of thio-glycolate to obtain a cell population containing a high proportion of macrophages¹². Finally, a population of spleen cells was depleted of B cells and macrophages using glass and nylon wool columns and the non-adherent cells (largely T cells) were treated with anti- θ and complement. A summary of the results obtained for AFP binding on these various cell populations is presented in Table 2. AFP binds to cells which are not adherent to nylon wool and are destroyed by anti- θ plus complement. No binding is seen with B-cell enriched populations (spleen treated with anti- θ plus complement) or in the spleens of nude mice. We cannot rule out a small amount of binding to macrophages but the major cell type involved seems to be a T cell. Figure 1 illustrates the surface binding of AFP by spleen cells.

Since only a fraction (about one-third) of the T cells bind AFP, it is possible that a subclass of T cells may be involved in AFP suppression. More sensitive techniques, such as the use of ¹²⁵I-AFP, will, however, be necessary to determine whether significant numbers of molecules of AFP bind to "negative" T and B cells but are not present in sufficient numbers to be detected by the fluorescence method.

The relatively small numbers of thymocytes (<1%) and

Table 1 Percentage surface staining for AFP in the primary and secondary lymphoid tissues*

Treatment	Spleen	Lymph node	Thymus	Bone marrow	Cortisone resistant† thymocytes	Nude mouse spleen cells
Media	< 1	< 1	< 1	< 1	< 1	< 1
NMS	< 1	< 1	< 1	< 1	< 1	< 1
MAF	18	23	< 1	2	6	< 1

* 15×10^6 viable cells incubated in media, normal mouse serum (NMS) at 2 mg ml⁻¹ or mouse amniotic fluid (MAF) at 2 mg ml⁻¹, for 90 min at 37 °C. Cells washed three times in cold media (RPMI 1640), suspended in 100 µl of media plus 50 µl of a fluoresceinated monospecific anti-AFP antiserum and incubated at 4 °C for 30 min. Surface fluorescence examined using a Leitz ortholux microscope. Figures are the average of duplicate experiments.

†Cortisone acetate (125 mg kg⁻¹) was injected intraperitoneally 2 d before the thymocytes were collected.

cortisone resistant thymus cells (6%) demonstrating binding compared with 18% of peripheral spleen T cells, suggest the possibility that AFP binding is detecting surface receptors which require additional maturation after peripheralisation from the thymus.

The binding of AFP to a T-cell population is compatible with the previous data from our laboratory showing that the addition of AFP late in the antibody response does not suppress the synthetic capacity of B cells⁴. We also reported^{5,6} that the IgA and IgG responses are more sensitive to the suppressive effects of AFP than are IgM responses. These findings are consistent with AFP exerting its effect on helper T cells since the switch from IgM to IgG and IgA is thought to be T-cell dependent^{13,14}. The observations⁷ that AFP has a direct effect on certain T cell dependent functions (mitogen and allogeneic stimulation) in mice is consistent with the findings presented here. Finally, preliminary experiments (R.J.D., R. H. Keller, and T.B.T., unpublished) indicate that concanavalin A (con A) and methyl- α -D-glucopyranoside inhibit AFP binding suggesting that the con A and AFP binding sites may be identical or closely spaced on the membrane surface.

Table 2 Percentage surface staining for AFP*

Treatment	Spleen cells	Spleen cells passed over nylon wool†	Peritoneal exudate cells
Media	< 1	< 1	< 1
NMS‡	< 1	< 1	< 1
MAF	18	34	4
Anti- θ + complement then MAF	2	4	ND
Anti-Fab + complement then MAF	38	ND	ND

*Figures represent the average of two separate experiments.

†Indirect immunofluorescence with anti- θ and anti-Fab showed that these preparations contained 88% θ -positive and 7% immunoglobulin-positive cells whereas the starting spleen cells contained 45% T and 44% B cells.

‡Normal mouse serum (NMS).

ND, Not done.

The mechanism of AFP suppression is unknown. T cells could be "turned off" by AFP binding to their surface, for example by mechanisms involving changes in cellular concentrations of cyclic AMP and cyclic GMP. Alternatively, AFP may activate suppressor cells or promote differentiation of a precursor T cell into a suppressor cell population. Further studies are necessary to define the mechanism(s) involved in AFP induced suppression.

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Hormonal regulation of α_1 foetoprotein

THE clinical usefulness of α_1 foetoprotein (AFP) is extensively documented¹ but the biological significance of this protein and the mechanisms regulating its synthesis remain unknown. We have investigated the effect of various hormonal, nutritional and haematological conditions on AFP metabolism in newborn and adult rats and mice. We present our results and some conclusions on regulation and function of AFP.

Different types of hormones markedly suppressed serum AFP levels in newborn rats. Among them, glucocorticoids (prednisolone 3 µg g⁻¹ d⁻¹, dexamethazone 2 µg g⁻¹ d⁻¹, hydrocortisone 15 µg g⁻¹ d⁻¹) all induced a dramatic decrease in blood AFP (Fig. 1) together with an increase in albumin and total protein levels; this effect was partly reversible on cessation of treatment. Adrenocorticotrophic hormone (ACTH) (10 U d⁻¹), levothyroxine (3 µg d⁻¹), adrenaline (1:10,000, 2 µl g⁻¹ d⁻¹) and db-cyclic AMP (10 µg g⁻¹ d⁻¹) also produced significant decreases in AFP levels without depleting albumin and total protein levels. Inconsistent or non-significant effects were observed with desoxycorticosterone (0.025 µg g⁻¹ d⁻¹), insulin (semilente 0.03 U g⁻¹ d⁻¹), glucagon (0.1 µg g⁻¹ d⁻¹), 17- β -oestradiol (3 µg g⁻¹ twice a week), testosterone (3 µg g⁻¹ twice a week) and progesterone (5 µg g⁻¹ three times a week). Neonatal adrenalectomy, ovariectomy, orchidectomy, radiothyroidectomy or thymectomy did not modify the normal physiological disappearance curve of AFP. Adrenalectomy suppressed the effect of ACTH and reduced the effect of thyroxine but did not modify the effects of prednisolone and db-cyclic AMP. Thyroidectomy did not modify the effects of prednisolone, db-cyclic AMP or thyroxine.

Metabolic studies were carried out on prednisolone-, thyroxine- and adrenaline-treated rats. These three hormones did not significantly alter the serum clearance of radiolabelled AFP (half life 23 h), although they all decreased incorporation of ¹⁴C-leucine in to AFP at a rate proportional to serum levels (Fig. 2). Liver tissue AFP content was also drastically decreased by prednisolone. These results indicate that prednisolone and probably adrenaline and thyroxine depress AFP levels through a selective blockade of its synthesis.

Attempts to correlate AFP levels with liver DNA synthesis activity of rats during uninterrupted prednisolone treatment disclosed the following facts. Prednisolone injection is rapidly followed by a marked depression of ³H-thymidine incorporation in liver parenchymal cells. This negative phase, however, is always followed by a burst of labelling activity 8 d after injections are started (Fig. 1). The newly labelled cells are typical hepatocytes uniformly dispersed throughout the lobule.

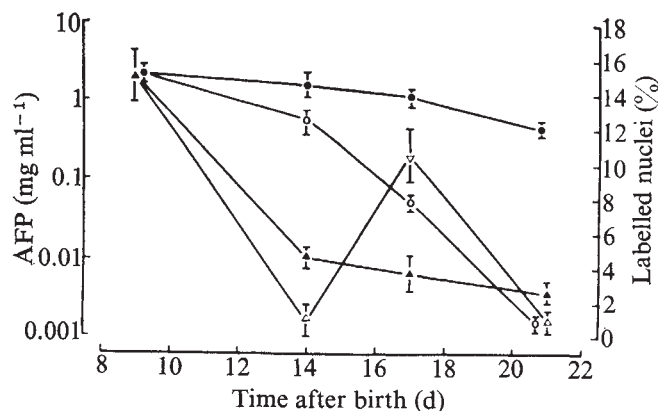
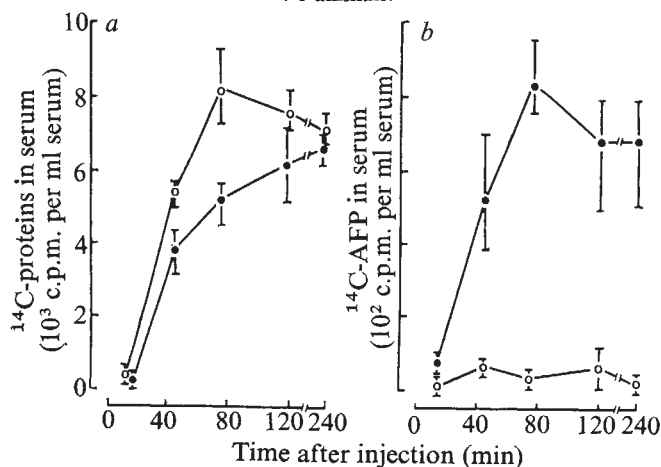


Fig. 1 Effect of prednisolone ($3 \mu\text{g g}^{-1} \text{d}^{-1}$ from days 9 to 21) on serum AFP levels (●, ○) and ^3H -thymidine incorporation in liver cells of newborn rats (▲, △). Mean $\pm$ s.d. (4–6 rats). ●, ▲, Controls; ○, △, prednisolone. AFP-suppressive action of prednisolone was effective throughout the postnatal period (0–21 d) and also before birth (injection into amniotic sac of 16-d pregnant rats). The experiments were carried out on Sprague-Dawley rats and C3H/HeB mice. Hormones injected were current pharmaceutical products; usually, intraperitoneal injections were used, in control animals receiving equivalent amounts of carrier. In neonatal experiments, half of every litter was used as control. Albumin and α_1 foetoprotein were quantitated by electroimmunodiffusion. The hepatic DNA synthesis activity was measured by autoradiography of liver tissue taken 1 h after intraperitoneal injection of ^3H -thymidine ($2 \mu\text{Ci g}^{-1}$). Protein synthesis activity was evaluated by measuring the amount of radioactivity incorporated in serum AFP and total proteins 15–240 min after intravenous or intraperitoneal injection of ^{14}C -leucine ($1.5 \mu\text{Ci}$ per 100 g). The AFP catabolic rate was determined by measuring the serum clearance of ^{125}I -labelled antigen (purified by the method of Nishi²) between 6 and 168 h after injection of radioactive material ($1 \mu\text{Ci}$ per animal).

This wave of DNA synthesis is never accompanied or followed by a resurgence of AFP secretion. It is in turn followed by another phase of suppression of ^3H -thymidine incorporation. Although the blocking action of glucocorticoids on liver DNA synthesis of newborn rats is a well known phenomenon³, to our knowledge, such a biphasic effect of prednisolone has not been reported so far.

Our data thus indicate that adrenal and thyroid hormones may have an important function in the physiological regulation of AFP metabolism. Further, assuming that AFP is produced by a liver parenchymal cell and considering the association of AFP synthesis with cell division⁴, the following conclusions may be reached. The secondary hepatocyte proliferation wave which appears during prednisolone treatment (Fig. 1) infers

Fig. 2 Effect of prednisolone ($3 \mu\text{g g}^{-1} \text{d}^{-1}$ for 3 d) on ^{14}C -leucine incorporation in total serum proteins (a) and serum AFP (b) of 8-d-old rats. Radioactivity recovered from 5% TCA precipitates and from AFP-anti-AFP precipitates. Mean and limit values for 4–6 animals.



that AFP synthesis is not a prerequisite for hepatocyte proliferation; moreover, it shows that AFP is not produced indifferently by any type of mitotic hepatocyte. The rapid labelling of circulating AFP after ^{14}C -leucine administration (Fig. 2) indicates that AFP is rapidly secreted into the blood following its synthesis. Therefore, that AFP is synthesised before mitosis and released after mitosis^{4–5} may be excluded; furthermore, injecting 4-d-old rats with colchicine ($25 \text{ ng g}^{-1} \text{d}^{-1}$ for 6 d) did not modify serum AFP levels (although it lowered by 47% the number of ^3H -thymidine-labelled liver cells).

In contrast to newborn rats (and mice), glucocorticoids usually have an enhancing effect on AFP levels in adult animals submitted to AFP-inducing conditions. This is best illustrated using mice intoxicated with CCl_4 (Fig. 3). In such animals, prednisolone markedly reduces the regenerative hepatocyte proliferation, but strikingly increases serum AFP levels; furthermore, the peaks of cellular proliferation and AFP secretion are shifted respectively from 40 to 72 h and from 3–4 to 6 d in control compared with hormone-treated animals (Fig. 3). Although prednisolone aggravates liver damage (as evidenced by levels of transaminases) and enhances passive release of AFP from injured cells, such opposite effects on AFP levels and cell proliferation clearly do not support the view that any dividing hepatocyte can resume AFP synthesis.

One interpretation would be that AFP synthesis can occur only during a definite stage of liver parenchymal cell ontogeny—in other words that AFP is the exclusive product of a 'transitional' cell, mitotically active and lying ontogenically between some stem cell (the 'oval' cell⁶) and the fully mature hepatocyte. This view is in very close agreement with the

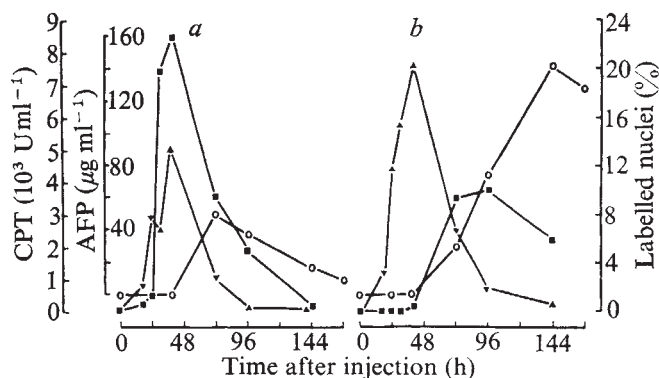


Fig. 3 C3H male adult mice injected intraperitoneally at 0 h with 0.1 ml CCl_4 per 100 g and from day 0 with daily subcutaneous injections of prednisolone (0.5 mg per animal) or saline (a). Each point is the mean of 4–6 animals. ▲, Cellular proliferation time (CPT); ○ AFP; radioactivity.

findings and hypotheses of Onoe *et al.* (see also Abelev⁷), and is supported by the work of Guegen *et al.* These authors have found that in newborn rat liver cell cultures, glucocorticoids promote the proliferation of mature hepatocytes but block (reversibly) the proliferation of 'intermediary cells'. If one assumes that such an intermediate cell is the AFP producer, this *in vitro* situation would account for the kinetics of AFP observed in newborn rats treated with prednisolone.

This could explain the lag phase between the hepatocyte division wave and peak of AFP production during liver regeneration. Being dependent on concomitant recruitment of progenitor cells, AFP production would take place during a later phase of liver repair, that is, during a fugacious intermediate mitotic stage of the stem cell on its way towards the mature hepatocyte. Thus, the effect of prednisolone on AFP levels during CCl_4 intoxication would be the result of a balance between strong indirect stimulation of stem cell recruitment due to enhanced liver damage and to reduced regenerative capacity of the hepatocytes and, as in the newborn, direct repression of the AFP-producing cell. At over 1 mg per animal

per d, the AFP-enhancing effect of prednisolone progressively decreases in CCl_4 -treated mice, which would fit this scheme.

We also found that prednisolone stimulates AFP production in tumour (3'-Me-DABu-induced)-bearing rats. AFP production of rat hepatoma is related to tumour growth rate⁹ and this enhancing effect of prednisolone is probably part of the representative response of tumours to glucocorticoids which seems to reflect the growth of the tissue¹⁰.

We conclude that physiological regulation of AFP is influenced by all factors, such as hormones, which control and influence the growth or the maturation of the liver and, more specifically, the proliferation and the differentiation of the intermediate pre-hepatocyte cell. Therefore, all conditions which modulate or interfere with general physiological maturation processes could also affect AFP metabolism. One such hindering situation is neonatal iron deficiency, the effect of which on serum AFP levels is depicted in Fig. 4.

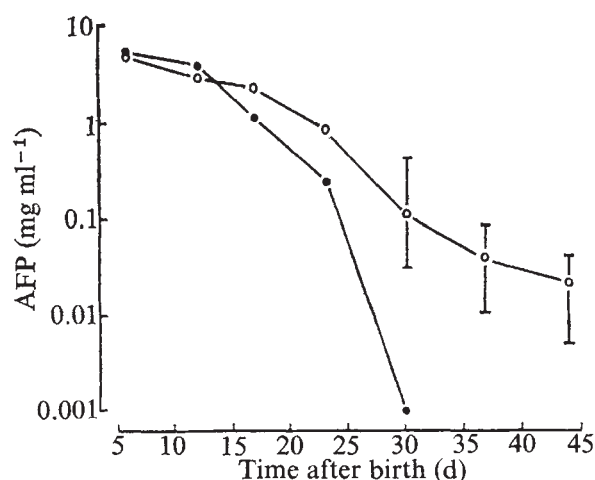


Fig. 4 Effect of iron deficiency on AFP levels of newborn rats. Deficiency was induced by serially bleeding gestating rats and by submitting them to an appropriate diet; weaned rats were maintained on the same diet. ●, Controls; ○, iron-deficient. Each point is the mean of 4–7 animals taken from 2–6 litters. Where indicated, bars represent limit values.

There are obvious analogies between albumin and AFP. The two proteins share similar physicochemical properties, such as molecular weight, isoelectric point and electrophoretic mobility, and both have dye-binding properties¹¹ and hormone carrier functions¹². The biological significance of AFP could be that of a foetal 'emergency' albumin, produced by a transitional parenchymatous cell committed to liver function but restrained in mitotic activity.

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Functional evidence for postsynaptic supersensitivity of central noradrenergic receptors after denervation

THE development of postsynaptic or postjunctional supersensitivity in peripheral, noradrenergically innervated tissue after deafferentation has been clearly demonstrated^{1–3}. In addition there exists considerable functional and biochemical evidence that central dopaminergic receptors in the neostriatum become supersensitive after lesions of the nigro-neostriatal projection^{4–8}. Neurochemical evidence has been provided which is consistent with the development of postjunctional supersensitivity of central noradrenergic receptors after deafferentation. Thus after intraventricular injections of 6-hydroxydopamine (6-OHDA), a neurotoxin for catecholaminergic neurones, noradrenaline (NA) stimulated synthesis of cyclic AMP is potentiated^{9–11}. Functional evidence for the development of this type of supersensitivity in central NA receptors is, however, lacking. An enhanced response to intracisternally administered α -methylnoradrenaline in 6-OHDA pretreated rats has been reported¹² but such an effect could be mediated by changes in either pre- or postsynaptic mechanisms. Our experiments were designed to provide functional confirmation for the presence of postjunctional supersensitivity in central noradrenergic receptors after lesions of central noradrenergic neurones. Clonidine [2-(2,6-dichlorophenylamino)-2-imidazoline] is a directly acting α -NA receptor agonist^{13–15} and has been shown to lower body temperature by a central α -noradrenergic mechanism. Thus intraperitoneal, intracisternal or intrahypothalamic injections of clonidine decrease body temperature^{16,17}. It was hypothesised that if central noradrenergic neurones become supersensitive after destruction of noradrenergic afferents by 6-OHDA, then clonidine-induced hypothermia should be potentiated in lesioned animals.

Rats which received stereotaxic 6-OHDA injections which lesioned both dorsal and ventral noradrenergic projections (Fig. 1) did not differ significantly from controls on baseline (predrug) colonic temperature (control group $37.05 \pm 0.17^\circ\text{C}$; experimental group $37.3 \pm 0.21^\circ\text{C}$), although there was a tendency for the lesioned group to have slightly raised colonic temperature. The mean temperature of the lesioned group was lower than controls after each dose of clonidine, but this failed to reach statistical significance. Highly significant differences were, however, obtained when the temperature decreases (baseline minus postdrug temperature) were compared in the two groups. The hypothermic responses to several doses of clonidine in control and lesioned animals are given in Fig. 1. At each dose, clonidine produced a significantly greater drop in colonic temperature in the lesioned animals than in controls ($P < 0.01$). At the lowest dose administered (0.05 mg kg^{-1}), clonidine did not significantly reduce colonic temperature in the control group but did produce significant hypothermia in the lesioned animals ($P < 0.01$). Ten days after the last clonidine injection the animals were killed by cervical fracture and the brains were dissected into hypothalamus, hippocampus and cerebral cortex. Cortex and hippocampus were combined and NA was measured in each sample¹⁸. Control NA values were: hypothalamus $2.07 \pm 0.03 \mu\text{g g}^{-1}$ and hippocampus-cortex $0.24 \pm 0.01 \mu\text{g g}^{-1}$. The 6-OHDA lesions reduced hypothalamic NA to 26% and combined hippocampal-cortical NA levels to 16% of control levels. These data indicate that the 6-OHDA produced extensive damage to both the dorsal and the ventral noradrenergic bundles²⁰. Although not measured in our present experiments, identical lesions do not significantly affect striatal dopamine levels^{21,22}.

Because these resulted in significant damage to two of the

major ascending noradrenergic bundles, an additional experiment was done in an attempt to determine whether the increased hypothermic response to clonidine was a consequence of damage to the ventral or the dorsal NA projection. The surgical procedure was the same as above except that one group of animals ($n = 8$) received 6-OHDA lesions ($4 \mu\text{g}$ per $2 \mu\text{l}$) of ventral noradrenergic bundle ($A, -1.4$; $L, \pm 0.9$; and $DV, +1.0$ mm; coordinates taken from stereotaxic zero), whereas others ($n = 8$) received lesions to the dorsal noradrenergic bundle ($A, +2.6$; $L, \pm 1.1$; and $DV, +3.7$ mm; coordinates taken from stereotaxic zero). The injection rate was $0.2 \mu\text{l min}^{-1}$. Control animals ($n = 8$) were sham operated. One month after the surgery, the animals were injected with clonidine-HCl (0.1 mg kg^{-1}) and colonic temperature was measured 120 min later. The baseline (preclonidine) temperatures of the three groups did not differ significantly although there was a tendency for the ventral bundle lesioned animals to be slightly hyperthermic (controls $37.04 \pm 0.17^\circ\text{C}$; dorsal noradrenergic bundle $37.0 \pm 0.11^\circ\text{C}$; ventral noradrenergic bundle $37.48 \pm 0.10^\circ\text{C}$). Clonidine significantly decreased rectal temperature in each group ($P < 0.01$). Compared with controls, however, only the animals receiving ventral noradrenergic bundle lesions showed a significant enhancement of the hypothermic response to clonidine ($P < 0.01$). The temperature decrease was $0.67 \pm 0.17^\circ\text{C}$ in controls, $0.75 \pm 0.28^\circ\text{C}$ in the dorsal bundle lesioned group and $1.30 \pm 0.11^\circ\text{C}$ in ventral bundle lesioned rats. The biochemical results of this experiment are given in Table 1. Ventral noradrenergic bundle lesions reduced hypothalamic NA by 89%, but did not have a statistically significant effect on hippocampal-cortical levels of NA. Lesions aimed at the dorsal noradrenergic bundle on the other hand reduced levels of hippocampal-cortical NA by 94% and hypothalamic NA by 69%.

Our results indicate that central noradrenoceptors, like central

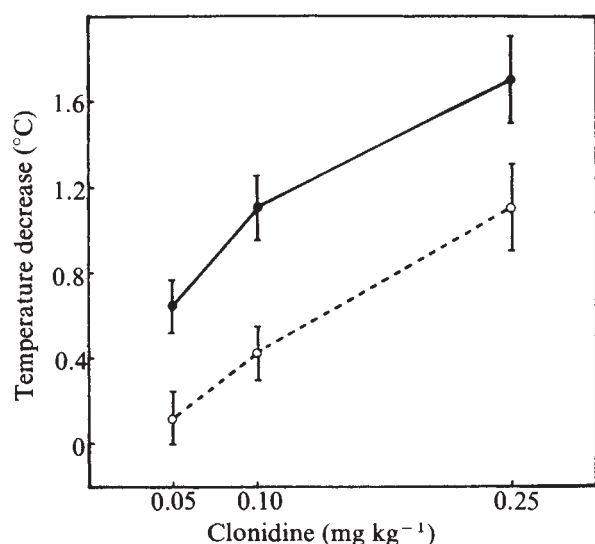


Fig. 1 Effect of clonidine-HCl on body temperature in controls (○) and in rats receiving 6-OHDA lesions of ascending noradrenergic projections (●). The 6-OHDA injections were aimed at the ascending noradrenergic projections caudal to the substantia nigra in male Wistar rats (280–320 g). The coordinates were: $A, +1.0$ mm; $L, \pm 1.3$ mm; and $DV, -2.1$ mm according to König and Klippel¹⁸. 6-OHDA hydrobromide was injected bilaterally ($4 \mu\text{g}$ in $2 \mu\text{l}$, dosage expressed as the base, dissolved in 0.15 M saline containing 0.2 mg ml^{-1} ascorbic acid) through a 34 gauge needle at a rate of $0.4 \mu\text{l min}^{-1}$. Control animals underwent sham operations. Three months after the surgery, both groups received intraperitoneal injections of clonidine-HCl in the order 0.1, 0.05 and 0.25 mg kg^{-1} (dosage expressed as the salt) with 4 d between each drug administration. Colonic temperature was measured before and 120 min after the injection and was determined by inserting a probe 4 cm into the rectum. Ambient temperature was $22\text{--}24^\circ\text{C}$. Data represent means $\pm$ s.e.m. of 8 animals in each group. The groups differed significantly at each dose ($P < 0.01$).

Table 1 Effect of 6-OHDA lesions of the dorsal or ventral noradrenergic bundles of hypothalamic and hippocampal-cortical levels of NA

	Hypothalamic NA ($\mu\text{g g}^{-1}$)	Hippocampal-cortical NA ($\mu\text{g g}^{-1}$)
Controls	2.17 ± 0.08	0.24 ± 0.01
Dorsal noradrenergic bundle lesion	$0.66 \pm 0.06^*$ (31%)	$0.014 \pm 0.01^*$ (6%)
Ventral noradrenergic bundle lesion	$0.24 \pm 0.04^*$ (11%)	0.21 ± 0.01 (85%)

Data represent means $\pm$ s.e.m. of 8 animals in each group. Numbers in parentheses indicate percentage of control values.

*Significantly different from controls ($P < 0.01$).

dopamine receptors, undergo changes after deafferentation which are consistent with the postjunctional supersensitivity hypothesis. Thus, the dose-response curve of the hypothermic response to clonidine, a noradrenoceptor agonist, was shifted to the left in animals bearing lesions of at least two of the major ascending noradrenergic projections (Fig. 1). Such a shift in the dose-response curve has been taken as a primary indicator of postjunctional supersensitivity¹⁻³. Reid²³ found that the hypothermic effect of clonidine (0.5 mg kg^{-1}) was not significantly increased 7–12 d after intracisternal injections of 6-OHDA, although there was a tendency for the experimental animals to show a greater drop in temperature. The reason for this discrepancy is not clear but may result from differences in procedure, or it may be that Reid did not obtain sufficient reductions in NA. Another criterion for postjunctional supersensitivity is that the enhanced response has a gradual onset and increases with time. Further studies will be required to determine if the enhanced hypothermic effect of clonidine satisfies this second criterion.

In the second experiment, an attempt was made to determine to what extent lesions of the dorsal or ventral noradrenergic bundles contributed to the observed effects. Although the lesions were not completely effective in destroying one system without damaging the other, reasonably discrete lesions were obtained (Table 1). It is obvious from the results of this experiment that the potentiation of clonidine-induced hypothermia was a consequence of damage to the ventral noradrenergic bundle. These data suggest, therefore, that clonidine produces hypothermia by an action on central noradrenoceptors which are normally innervated by the ventral noradrenergic bundle. They also suggest that this ascending system, which terminates mainly in the hypothalamus²⁰, may have an important role in thermoregulation. It would be of interest to determine to what extent animals with lesions of the ventral noradrenergic bundle may have thermoregulatory deficiencies.

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Spontaneous voltage fluctuations in retinal cones and bipolar cells

TRIFONOV¹ and others have suggested that vertebrate rods and cones release transmitter continuously in the dark and that the effect of light is to suppress this release by making the inside of the cell more negative. On this hypothesis the bipolar cells, which receive information from cones, should be electrically noisy in the dark, because of random fluctuations in the release of cone transmitter, and relatively quiet in the light when the release of transmitter is suppressed².

Such an effect is illustrated in Fig. 1a, which is an intracellular recording from a hyperpolarising bipolar cell in the isolated eyecup of the turtle, *Pseudemys scripta elegans*. In darkness, the cell voltage fluctuated at low frequency in an apparently random manner over a range of about 5 mV peak-to-peak. Steady illumination of the centre of the receptive field produced a maintained hyperpolarisation of about 6.5 mV and a considerable reduction in noise. When the light was turned off, there was a brief depolarising transient of almost 30 mV followed by slow recovery of both dark voltage level and noise. The voltage variance was 1.53 mV² in darkness and 0.045 mV² in light.

The possible order of magnitude of the events underlying the dark noise of a bipolar cell may be estimated as the change in voltage variance divided by the change in mean voltage⁷ and is found to be 0.24 mV. This is similar to the amplitude of a miniature end-plate potential (e.p.p.), but the ionic movement underlying the event must be much smaller than that in a miniature e.p.p. since the input resistance of bipolar cells is likely to be two or three orders of magnitude higher than that of a muscle fibre. At present it is uncertain whether the hypothetical events underlying the bipolar cell noise are analogous to the effects produced by single acetylcholine molecules or to those of the packets of molecules which produce miniature e.p.p.s.

There was often no significant and reproducible change in variance when the bipolar cell depolarised in response to an annulus of light, but in some cells a consistent reduction in noise did occur. This is illustrated in Fig. 1b, where the dark variance of 0.072 mV² was reduced to 0.029 mV². Since depolarisation of bipolar cells by an annulus is mediated by hyperpolarisation of luminosity horizontal cells^{3,6}, these findings suggest that the horizontal cells liberate hyperpolarising transmitter at a relatively high rate in darkness and at a lower rate when hyperpolarised.

Figure 2 illustrates the unexpected result that a steady light suppresses noise in cones as well as in bipolar cells. The record in Fig. 2a was obtained on a red-sensitive cone, which from its small receptive field was taken to be an isolated cone not coupled to its neighbours⁸. The cell was unusually noisy and the dark variance of 0.40 mV² was reduced to 0.025 mV² during illumination which hyperpolarised by about 14 mV at the initial peak. Effects of this kind were observed consistently, although the cells were usually less noisy than in the example given. A more typical result was obtained from a green-sensitive cone (Fig. 2b) in which light reduced the dark variance of 0.018 mV² to

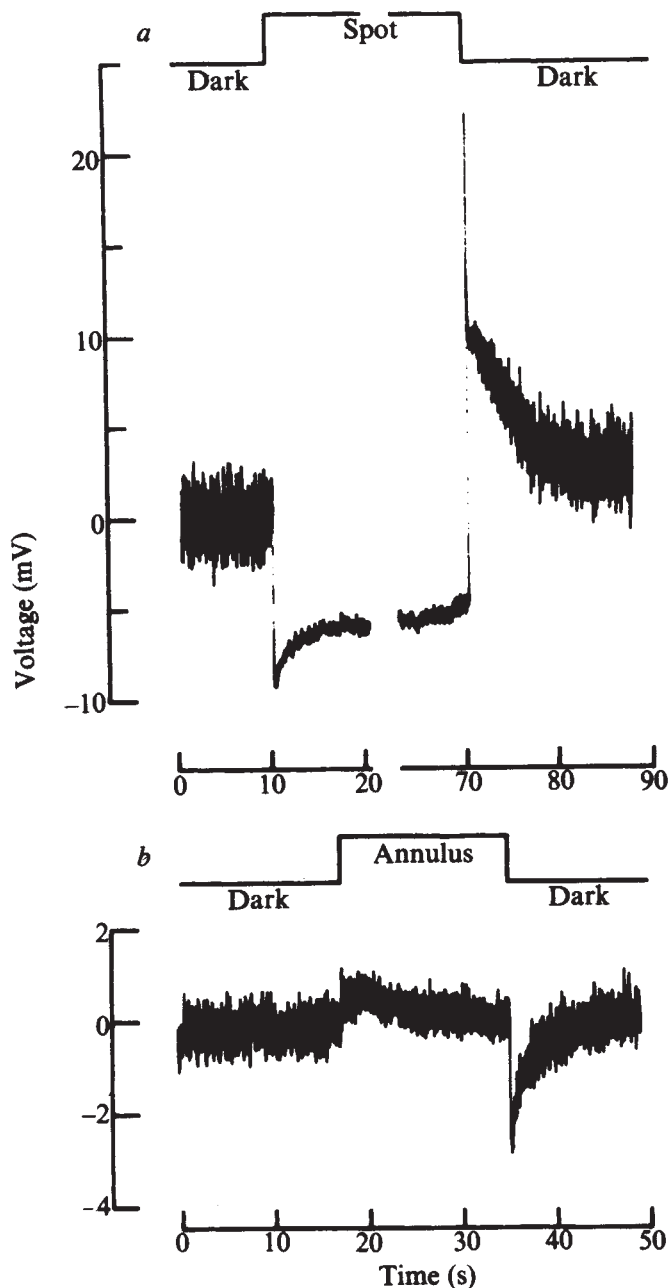


Fig. 1 Intracellular recordings from centre-hyperpolarising, red-sensitive bipolar cells. Abscissae measure time and ordinates give membrane potential relative to mean voltage in darkness (called zero). *a*, Response to a 210- μ m diameter centred spot which delivered 4.13×10^4 photon $\mu\text{m}^{-2} \text{s}^{-1}$ to the retina at 639 nm; *b*, Response of another cell to an annulus of 630 μ m internal diameter and 1,300 μ m external diameter; photon flux was the same as in (*a*). The relatively low variance and small light response are attributed to damage by the impaling micro-electrode. A PDP-11 computer (DEC) was used to digitise the signals and calculate the variances; dark values given in the text are the mean of measurements taken before and after illumination. 1,024 points were sampled at 5 ms intervals after passing the signal through an RC high-pass filter with 1 s time constant and a 3-pole low-pass filter with 50 Hz cutoff frequency. Micropipettes were drawn from capillary tubing containing a fused partition ("0 tubing"), as they were found to fill consistently and to show sufficiently low intrinsic noise. Bipolar cells and cones (Fig. 2) were identified by electrical criteria established from dye marking experiments³⁻⁶.

0.004 mV² or less (less because much of the noise in the light may be instrumental in origin). Since the spatial properties of cells with low electrical noise indicate that they are coupled electrically to neighbouring cones, it seems likely that coupling

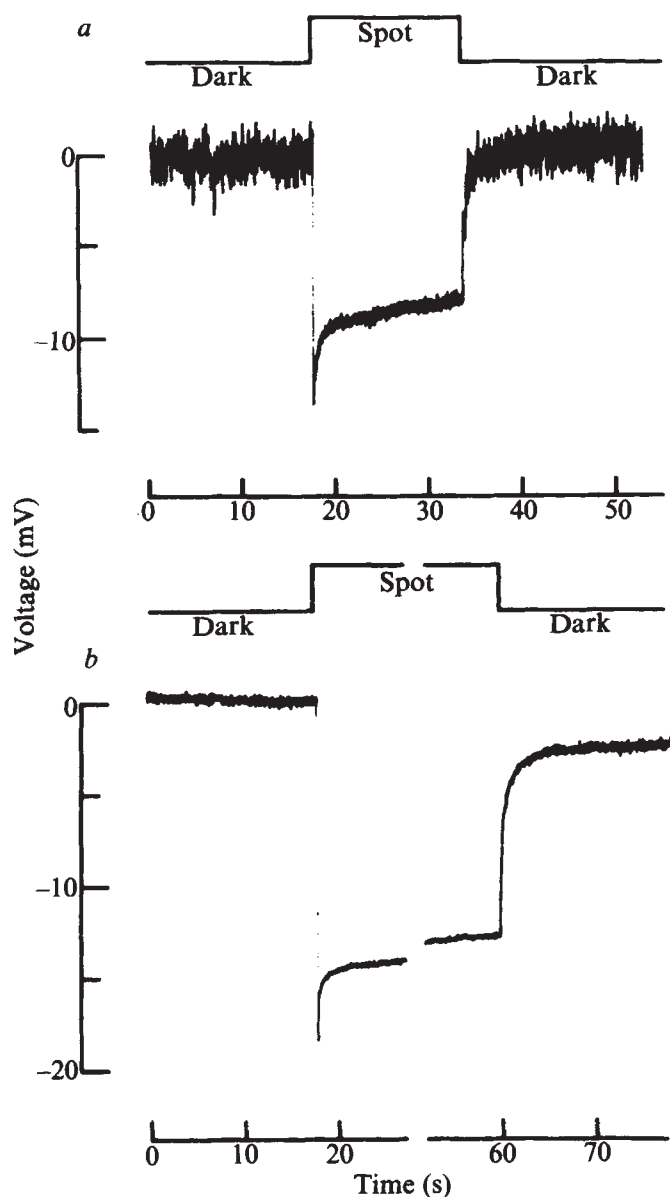


Fig. 2 Recordings from cones in darkness and during steady central illumination. *a*, Response of an isolated red-sensitive cone to a 6- μm spot delivering 8.05×10^5 photon $\mu\text{m}^{-2} \text{s}^{-1}$ at 639 nm; *b*, Response of a green-sensitive cone to a 132- μm spot delivering 1.67×10^6 photon $\mu\text{m}^{-2} \text{s}^{-1}$ at 559 nm. The relatively low noise in the dark is characteristic of coupled cones.

reduces noise (see refs 9, 10). Lights which hyperpolarised by more than a few mV consistently reduced noise, but very dim lights gave no significant change and it is uncertain whether the initial slope of the curve relating noise to light intensity is positive or negative.

It is surprising to see noise suppression in a cone, because the natural expectation is that random absorption of quanta should give 'photon noise' as it does in a photomultiplier tube or in an invertebrate photoreceptor¹¹. The synaptic action of horizontal cells on cones⁵ might contribute to the variance in the dark, but this cannot account for the main observation because small areas of illumination (Fig. 2) suppress the noise even though their effect on horizontal cells is very small. It therefore seems necessary to suppose that there is a source of electrical noise in the dark which is reduced as the cone is hyperpolarised by light and more than compensates for the fluctuations introduced by the random absorption of light quanta. A possible

source of "dark noise" would be random closure of the light-sensitive ionic channels either spontaneously or as a result of variations in the concentration of a blocking or opening substance. In the kinetic model of Baylor *et al.*¹², it is assumed that light releases a blocking molecule which hyperpolarises the cell by closing ionic channels. If the relationship between hyperpolarisation (u) and the concentration (z) of the blocking molecule is of the form

$$u/u_{\text{max}} = z/(z + K)$$

and if the variance of z is proportional to z , then the variance of u should be maximal when $z = K/3$ and $u = u_{\text{max}}/4$ (ref. 7). In terms of this hypothesis, our data require that the dark value of z be about $K/3$ and that the cell be hyperpolarised in darkness by about 5 mV from the potential it would have if all the light-sensitive ionic channels were open all of the time.

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Evidence that sensory axons are mitogenic for Schwann cells

We have obtained preparations of peripheral nerve tissue in culture which provide either Schwann cell populations, essentially free of connective tissue cells such as fibroblasts, or outgrowing neurites from sensory (or autonomic) ganglia entirely free of ensheathing Schwann cells or connective tissue elements. Thus, these two major elements of peripheral nerve can be studied and characterised separately or in recombination. We report here that the 'bare' sensory ganglion neurite provides a potent stimulus for thymidine incorporation and mitosis in Schwann cells.

Tissue culture techniques utilised in obtaining separation of these two components from the rat peripheral nervous system will be reported in detail elsewhere. Briefly, to obtain Schwann cells, rat dorsal root ganglia (DRG) obtained just before birth were stripped of their capsule and after explantation were exposed to cytosine arabinoside (ara-C)¹ and fluorodeoxyuridine (FdU)². Cultures were fed with medium containing these compounds at 10^{-5} M concentrations during days 1 and 3 *in vitro*, and with regular medium during day 2 and subsequently. After 10 d *in vitro* ganglia were transplanted to new culture dishes where they provided a fresh outgrowth of neurites and Schwann cells within 5-7 d, and this outgrowth was essentially free of connective tissue cells. The neurites could then be easily eliminated from these cultures by removing the ganglionic mass containing the neurone somata (Fig. 1a). After neurite breakdown the population of Schwann cells previously related to the neurites was retained. This population

is hereafter referred to as a Schwann cell bed. To obtain unensheathed neurites, the schedule of exposure of DRG to ara-C and FdU was continued through day 7 *in vitro* in alternation with the regular medium as described above. In addition, after day 7 the cultures were maintained on medium containing FdU, which suppresses subsequent Schwann cell proliferation. After about 1 month of continued exposure to FdU, Schwann cells could not be detected in the outgrowth arising from the ganglion. The ganglion could then be transplanted to a new dish where it provided a fresh growth of neurites completely free of Schwann cells in the absence of antimitotic agents. These cultures are hereafter referred to as bare neurite preparations.

Schwann cell beds prepared as described above may contain fibroblasts in some cases. In the experiments to be described below the incidence of fibroblasts was so infrequent that they did not interfere with the observations. In addition, fibroblasts in culture are easily distinguished from Schwann cells on the basis of their larger size, flatter shape and lack of specific relationship to neurites³. We have observed that with two additional 1-d exposures to ara-C and FdU it is possible to completely eliminate fibroblasts from the cultures, and to obtain populations of Schwann cells virtually free of fibroblasts. Our general culture technique uses a collagen substrate in a modified Petri dish, a medium containing 25% human placental serum and 10% 9-d chick embryo extract (50% in saline), nerve growth factor, and a 5% CO₂-95% air atmosphere⁴.

Autoradiography after application of ³H-thymidine (0.5 μ Ci ml⁻¹ in regular medium) to Schwann cell beds on successive days after ganglion removal demonstrates a decreasing number of labelled Schwann cells over a period of about 10 d; after this time less than five cells per hundred are labelled. It is then possible to transfer ganglia providing bare neurites into these culture dishes, both in areas occupied by Schwann cells and in areas free of Schwann cells. In more than ten separate experiments of this type we have consistently observed that: the presence of a ganglion providing bare neurites located remote from the Schwann cell bed has no effect on the Schwann cell labelling index; Schwann cells in beds only partly invaded by neurites exhibit a stimulation of ³H-thymidine incorporation

only in those bed regions directly invaded by bare neurites (Fig. 1, *a-c*); the degree of stimulation provided by bare neurites is dramatic, with about 90% of the Schwann cells in the invaded area incorporating ³H-thymidine after exposure for 42 h. The number of Schwann cells greatly increases in areas invaded by neurites and frequent mitotic figures are seen. Ganglia providing bare neurites not placed near Schwann cell beds do not generate a Schwann cell population, indicating that the implanted ganglia did not obtain this capability during the course of the experiment.

One of these experiments in which a ganglion providing bare neurites was placed along the border of an extensive Schwann cell bed is shown in Fig. 1. The labelling index is greatly increased in the zone of interaction between neurites and Schwann cells, but not elsewhere. The neurite population on the opposite side of the implanted ganglion is, at this point, essentially cell-free, but in preparations kept for a week or more after this stage Schwann cell invasion of the entire neurite halo occurs. The identity of these cells as Schwann cells is based not only on their general size and morphology (Fig. 1*b* and *c*) but also on electron microscopic observations of their distinctive ensheathing relationship to the axon.

The literature on Schwann cell proliferation during development and nerve injury contains many instances where the proliferative capability of the Schwann cell population is documented (reviewed in ref. 5). It is clear that Schwann cell proliferation in damaged nerve has often been ascribed to the presence of degeneration products within the nerve; the growing axon has not generally been considered a factor in Schwann cell proliferation; and there has been little discussion of the possible mechanism by which Schwann cell numbers are controlled during embryonic development. Our observations indicate that the presence of a growing nerve fibre during either initial growth or regeneration must be considered a potent stimulus to Schwann cell proliferation; there may, of course, be other stimuli.

The demonstrated mitogenic capacities of the axon may explain several recent *in vivo* observations. Thomas⁶ has observed that repeated crushes (which enable optimal axon

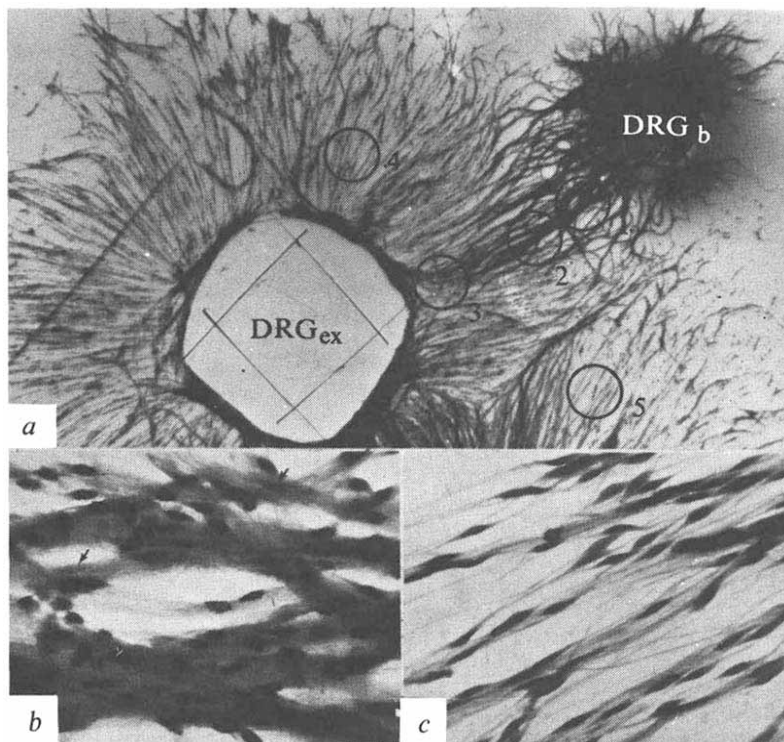


Fig. 1 *a*, Autoradiogram illustrating interaction between bare neurites and quiescent Schwann cells. Schwann cells derived from a dorsal root ganglion explant extend radially from the vacated site of the excised ganglionic mass (DRG_{ex}). A second ganglion (DRG_b) from which Schwann cells had been eliminated (by previous treatment with ara-C and FdU) was implanted in an area adjacent to the Schwann cell bed 8 d after excision of the first ganglion. Within 3 d bare neurites from the second ganglion (DRG_b) invaded the Schwann cell bed and the culture was exposed at that time to ³H-thymidine for 42 h. The culture was then fixed with 4% formaldehyde in phosphate buffer, processed for whole mount autoradiography using Kodak NTB2 emulsion, and stained with erythrosin β -Toluidine blue stain. At this low magnification the Schwann cell nuclei appear as small specks with the Schwann cell processes aligned in a radial manner from the zone marked DRG_{ex}. Darkly stained processes originating at DRG_b interact with and become unensheathed by Schwann cells derived from DRG_{ex} but remain unensheathed, at this early stage, on the opposite side of the ganglion. Counts of labelled and unlabelled Schwann cell nuclei were made in the fields designated by the circles. Counts were: 1, 115 (labelled)/15 (unlabelled); 2, 31/71; 3, 0/120; 4, 0/129; 5, 1/59. $\times 18$. *b*, Higher magnification of field 1 shown in *a*. This field clearly lies in the zone of interaction between Schwann cells and bare neurites. Most of the detectable Schwann cell nuclei in this area are covered with silver grains; only a few unlabelled nuclei (arrows) are present. $\times 204$. *c*, Higher magnification of field 4 shown in *a*. In contrast to *b* this field has not been invaded by bare neurites from the implanted DRG and none of the nuclei is labelled. In general the labelling index in quiescent Schwann cell beds is less than 5%, compared with a labelling index of more than 80% in areas where proliferation is stimulated by actively growing neurites. $\times 204$.

regrowth) of a peripheral nerve leads to a remarkable overproduction of Schwann cells in the distal nerve stump reaching a point at which these cells are applied in multiple layers around each single axon. Also, in crush lesions of the abnormal peripheral nerve roots of dystrophic mice, where a paucity of Schwann cells and myelin segments provides much less degenerating material than in normal nerve, an increase in the number of myelin segments and, presumably, Schwann cells is induced by crush injury⁷. The importance of the axon in provoking Schwann cell proliferation is also indicated by observations, on the proximal stump of severed or crushed sciatic nerve, that proliferation occurs independently of widespread nerve fibre or myelin degeneration and in conditions in which nerve regeneration is most actively occurring⁸.

Our observation of the axon-Schwann cell interaction resulting in mitogenesis illustrates one use of cultures containing either 'pure' Schwann cell populations or bare neurite preparations. These preparations may be useful in further analysis of the interaction between neurite and Schwann cell, as well as in studies of the basic properties of the Schwann cell itself.

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Biological reactivity of PVC dust

CONCERN has been expressed about the biological potential of polyvinyl chloride (PVC) and its associated monomer vinyl chloride monomer (VCM)¹. During the industrial processing of PVC, workers can be exposed to varying quantities of this material in the form of a dust. At the present time, however, there is little detailed biological or biochemical information on the effects of inhaled or ingested PVC, or on the reactivity of this dust material. The biological reactivity of other dusts (silica, asbestos) have been studied by a haemolysis technique² which is useful for assessing the degree of membrane-induced damage by a variety of toxic materials. Other *in vitro* screening systems using lung³ and other cells⁴ have also provided useful information on structural and biochemical changes induced by particulate matter. Here we report on the haemolytic potential of PVC dust, comparison of which is made with the highly haemolytic and biologically reactive chrysotile asbestos A (UICC standard reference sample) and the effect of PVC on lung fibroblast cultures.

The haemolysis technique used has been described in detail previously². The degree of haemolysis was expressed as a percentage of the totally lysed sample and the results presented as the means and ranges (if any) of, at least, quadruplicate assays. Two samples of PVC were tested (KM 1 and KM2) both of which were obtained as finely-divided dried powders which had been formed by standard processing procedures for use in fabrication work.

The first sample (KM 1) was highly haemolytic at relatively low concentrations, 100% haemolysis (over 50 min) being achieved by between 7.5 and 10 mg of dust (Fig. 1a). With increasing concentrations of PVC, haemolysis seems to be reduced, but this effect is most likely the result of some of the released haemoglobin binding to the dust, which is then spun down into the pellet. Sample KM 2 PVC was found to be practically non-

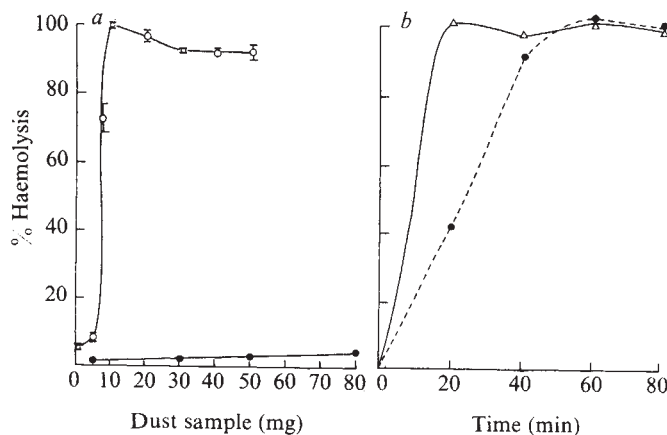
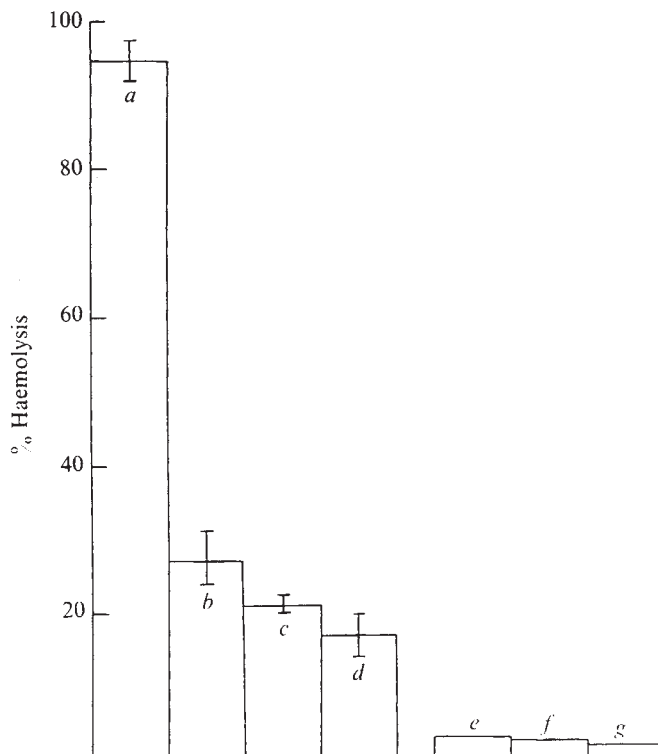


Fig. 1 Haemolysis by PVC dusts and chrysotile asbestos. *a*, Haemolytic potency of KM 1 (○) and KM 2 (●) PVC samples after 50 min; *b*, change in haemolysis with time by 7.5 mg samples of UICC chrysotile asbestos A (△) and KM 1 (●) PVC. Briefly, a 1% (v/v) suspension of packed rabbit erythrocytes in veronal buffered saline, pH 7.4, was used in all the experiments. The incubation mixture normally consisted of dust sample with 1 ml 1% erythrocyte suspension + 3 ml veronal buffered saline. After incubation and agitation for 50 min (or varying time intervals) at 37 °C the samples were centrifuged at 2,000 r.p.m. for 20 min and the extinction of the supernatant read at 541 nm. Controls consisting of a totally lysed sample (1 ml 1% erythrocyte suspension + 3 ml water) and a fragility control (1 ml 1% erythrocyte suspension + 3 ml veronal buffered saline) were treated identically in each experiment.

haemolytic. A sample of 100 mg of KM 2 PVC gave an equivalent haemolytic effect to 1 mg of sample KM 1. The haemolytic activity with time of a sample of KM 1 PVC (7.5 mg) was compared with an equivalent mass of chrysotile asbestos A (Fig. 1b). It is evident that the asbestos is a faster haemolytic agent, although total lysis is achieved by PVC after 1 h.

During the processing of different forms of PVC, a variety

Fig. 2 Haemolysis by washed samples of KM 1 PVC dust (7.5 mg) and the resulting supernatant fluids from these washings compared with the haemolytic activity of the untreated dust sample. KM 1 PVC *a*, Untreated; *b*, washed once; *c*, washed twice; *d*, washed three times; *e*, wash 1; *f*, wash 2; *g*, wash 3.



of agents are used which could contribute to the haemolytic effect of the dust. Therefore, samples (7.5 mg) of KM 1 PVC were washed once, twice and three times (5 s each wash, in 3 ml veronal buffer on a whirlimix; followed by centrifugation, 2,000 r.p.m. for 20 min) and the haemolytic potency of the washed dust samples and the supernatant fluid were compared with the lytic potential of an untreated PVC sample (Fig. 2). After a single wash, the haemolytic potency of KM 1 PVC is reduced by over 60% and subsequent washes reduce the activity of the dust even further. Thus the removal of some surface-associated material, which must exist in a reasonably soluble form, considerably reduces the biological potency of this PVC dust. It is also evident that this material, once removed from the dust surface and diluted out in solution, has very limited haemolytic activity (Fig. 2).

The effect of KM 1 PVC was studied on the levels of cell mat DNA, RNA, protein and hydroxyproline (assessment of collagen) in lung fibroblast cultures maintained *in vitro* for 24 d. The methods of isolation and cell culture and the analyses for DNA, RNA protein and hydroxyproline have been detailed previously^{3,5}. Before addition of different concentrations of KM 1 PVC (50–200 $\mu\text{g ml}^{-1}$ culture medium or 0.5–2.0 mg per culture) the dust sample was first washed in a balanced salt solution containing antibiotics (see legend to Fig. 3). Other methods of dust sterilisation (heat, autoclaving, radiation)

forms of PVC dusts exhibit a high haemolytic potential because of the presence of a readily soluble, surface-associated agent. Exposure to this type of PVC dust may thus constitute an additional health hazard because of the increased biological activity of the dust. Work is in progress to determine the nature of the haemolytically-active agent by assessing a wide range of PVC dusts of which complete knowledge has been obtained of the chemical processing. The possibility that VCM is the active agent has been explored, but both samples tested were found to contain immeasurable amounts of VCM (<1 p.p.m.). Nevertheless, the present study indicates that the introduction of a washing procedure after the processing of KM 1 PVC dust would certainly reduce or abolish the haemolytic activity of this material.

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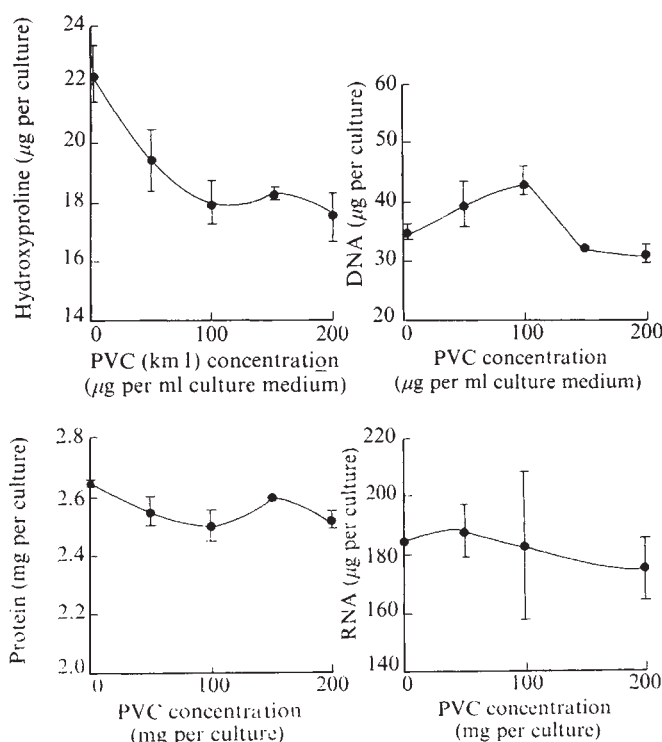


Fig. 3 The effect of different concentrations of KM 1 PVC on lung fibroblast cultures. The PVC sample was first washed with an antibiotic solution (penicillin (100 units) streptomycin (100 μg) in 1 ml) and then added as a single dose to 3-d-old cultures (in logarithmic growth). Cultures were maintained in 10 ml of 20% foetal bovine serum plus Waymouth's medium containing additional ascorbic acid⁵, changed twice weekly and removed for analysis on day 24.

were not considered to be feasible and while the washing procedure undoubtedly reduces the ability of this PVC sample to damage cell membranes (Fig. 2) fibroblast cultures treated with the dust all had lower levels of cell mat hydroxyproline after 24 d (Fig. 3). Some fluctuation in DNA levels was apparent with different dust concentrations but the significance of these is doubtful without further experimentation. There is little change in the level of total protein or RNA in the PVC-treated cultures.

From these preliminary findings we conclude that certain

Mechanism of induction of haemolytic anaemia by phenylhydrazine

A COMPOUND with the optical spectrum of a ferrihaemochrome was produced when ferricyanide-oxidised phenylhydrazine was added to a solution of ferrihaemoglobin¹. The three isomers of methylphenylhydrazine similarly resulted in ferrihaemochromes, but 4-hydrazinobenzoic acid did not². The induction of haemolytic anaemia by a substituted phenylhydrazine was related to the reactivity of its oxidised form with ferrihaemoglobin to produce a ferrihaemochrome^{3,4}. Further studies of the reaction of oxidised arylhydrazine with ferrihaemoglobin have established that the formation of a ferrihaemochrome-like product and the character of the optical spectrum of this product depend on the nature and position of substituents on the benzene ring of phenylhydrazine.

Substituted phenylhydrazine hydrochlorides were obtained from commercial sources or were synthesised by standard procedures. Each compound was purified by recrystallisation from 2 N HCl or ethanol, and the structure and purity of the recrystallised product were confirmed by its NMR spectrum, infrared spectrum, melting point, and analyses for C, H, N, and Cl. Oxyhaemoglobin solutions were deoxygenated by the passage of oxygen-free⁴ nitrogen or helium, and the resulting ferrohaemoglobin was oxidised to ferrihaemoglobin by the addition of excess ferricyanide. To a solution of ferrihaemoglobin in excess ferricyanide, a solution of arylhydrazine hydrochloride in water or ethanol was added, and the optical spectrum that resulted was recorded with the Cary Model 17 instrument. The spectra obtained with 3- and 4-chlorophenylhydrazine were similar to that previously reported with unsubstituted phenylhydrazine¹. The spectra obtained with 3,4- and 3,5-dichlorophenylhydrazine were distinguished by a more prominent absorption band in the red than the others. 2-Chloro- and 2,3-, 2,4-, and 2,5-dichlorophenylhydrazine resulted in spectra with a single broad maximum at around 540–550 nm and without a band in the red. 2,6-Dichlorophenylhydrazine, 2,6-dimethylphenylhydrazine, 2-hydrazinobenzoic

acid, and 4-hydrazinobenzoic acid each resulted in slow and incomplete formation of ferrihaemochrome. On the other hand, the ethyl ester of 4-hydrazinobenzoic acid resulted in the quantitative formation of a ferrihaemochrome. Results with the isomers of dichlorophenylhydrazine are shown in Fig. 1.

Arylhydrazine hydrochloride and potassium ferricyanide were mixed in oxygen-free solution, and the reduction of ferricyanide was followed at 420 nm (Fig. 2). In agreement with the stoichiometry of the oxidation of arylhydrazine to aryl-diazene^{6,8}, 2 mol of ferricyanide were rapidly consumed per mol of phenylhydrazine or 4-hydrazinobenzoic acid; further reduction of ferricyanide was very slow. 2,6-Dimethylphenylhydrazine, not shown here, also reduced two equivalents of ferricyanide very rapidly. 2,6-Dichlorophenylhydrazine, on the other hand, reduced ferricyanide more slowly, but after 10 min had consumed more oxidant than either phenylhydrazine or 4-hydrazinobenzoic acid.

The finding that each substitution on the benzene ring of phenylhydrazine resulted in a ferrihaemochrome with a different optical spectrum indicates that the ferrihaemochromes are compounds of ferrihaemoglobin with ligands that include the substituted benzene ring of the oxidised arylhydrazines and supports the suggestion that the optical spectrum obtained when oxidised phenylhydrazine was added to ferrihaemoglobin resulted from the binding of phenyldiazene ($C_6H_5N=NH$)¹. The bond was postulated to form between the iron atom of ferrihaem and the nitrogen atom bound to the benzene ring in the phenyldiazene molecule⁸. The low reactivity of 2,6-dichlorophenyldiazene and of 2,6-dimethylphenyldiazene can be explained by the steric hindrance of their respective di-*ortho* substituents to the formation of such a bond. The reactivity of oxidised ethyl 4-hydrazinobenzoate suggests that the low reactivity of oxidised 4-hydrazinobenzoic acid is due not to the ring position or size of the carboxyl group, but to its negative charge.

The effect of adding arylhydrazine to oxyhaemoglobin was examined with the compounds of Fig. 2. Phenylhydrazine

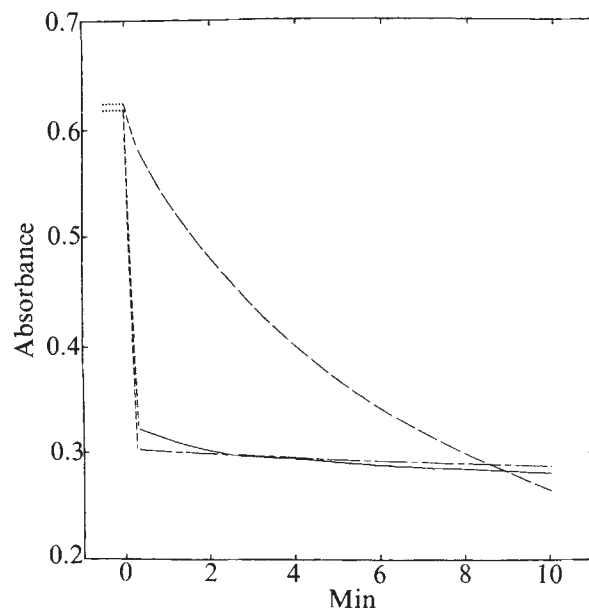
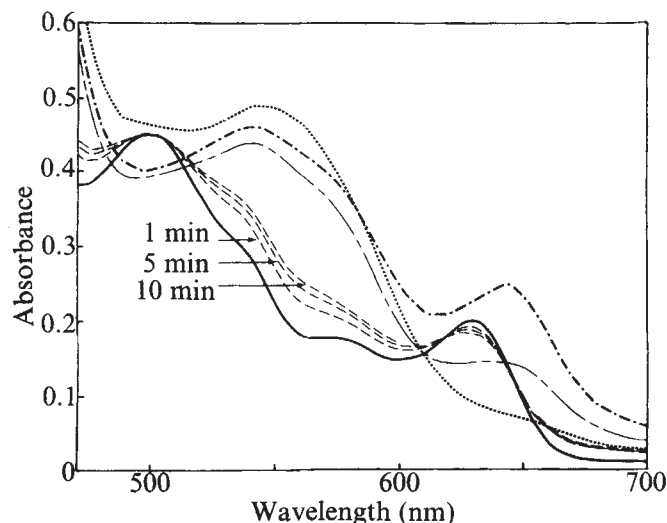


Fig. 2 Oxidation of arylhydrazines by potassium ferricyanide. The disappearance of the absorption peak of potassium ferricyanide at 420 nm was followed at 23 °C. Absorbance of 6.0×10^{-4} M potassium ferricyanide (.....). Interval between addition of arylhydrazine hydrochloride and beginning of absorbance record (-----). Absorbance after arylhydrazine hydrochloride was added to make 1.5×10^{-4} M solutions of: phenylhydrazine (—); 2,6-dichlorophenylhydrazine (— — — —); 4-hydrazinobenzoic acid (— — —).

caused a rapid change in colour to greenish brown followed by progressive precipitation of protein. 4-Hydrazinobenzoic acid resulted in complete replacement of oxyhaemoglobin by a mixture of ferrihaemoglobin (75%) and ferrihaemochrome (25%) without precipitation. 2,6-Dichlorophenylhydrazine resulted in slow transformation of oxyhaemoglobin to 15% ferrihaemoglobin after 2.5 h. Beaven and White⁷ reported that 2- and 4-nitrophenylhydrazine reacted with oxyhaemoglobin to produce precipitates of "green haemoglobin" but that 2,4-dinitrophenylhydrazine produced only ferrihaemoglobin. We found that 2- and 4-nitrophenylhydrazine reacted with ferrihaemoglobin in the presence of excess ferricyanide to form ferrihaemochrome-like compounds but that 2,4-dinitrophenylhydrazine did not. Substituted phenylhydrazines, including ethyl 4-hydrazinobenzoate, that resulted in the quantitative formation of ferrihaemochromes also induced haemolytic anaemia in experimental animals while 2- and 4-hydrazinobenzoic acid, 2,6-dichlorophenylhydrazine, and 2,6-dimethylphenylhydrazine did not^{2,8}. Thus, the ability of an arylhydrazine to cause both oxidative denaturation of oxyhaemoglobin and haemolytic anaemia in animals paralleled the ability of a product of its oxidation to react rapidly and quantitatively with ferrihaemoglobin to produce a ferrihaemochrome.

Phenylhydrazine results in the formation of ferrihaemoglobin from oxyhaemoglobin⁹, and phenylhydrazine is oxidised to phenyldiazene by oxygen⁵. The two products, ferrihaemoglobin and phenyldiazene, react rapidly and quantitatively with each other or form a ferrihaemochrome. All three reactions are essential because 4-hydrazinobenzoic acid, which was shown to take part in the first two but not the third of these reactions, did not result in oxidative denaturation or anaemia. An amino acid replacement in the haem pocket destabilises haemoglobin by breaking non-polar contacts between haem and globin¹⁰ and results in unstable haemoglobin haemolytic anaemia¹¹. The binding of phenyldiazene was postulated to destabilise haemoglobin in phenylhydrazine-induced haemolytic anaemia⁵; however, the acuteness of the latter anaemia contrasts with the chronic nature of the former. The production of free radicals and hydrogen peroxide in the reduction of molecular oxygen and the bound oxygen of oxyhaemoglobin by phenyl-

Fig. 1 Spectra of ferrihaemoglobin (—) and of ferrihaemochromes produced by oxidised dichlorophenylhydrazines: 2,3-dichloro (-----); 3,4-dichloro (- - - - -); 3,5-dichloro (— · — · —); 2,6-dichloro (— — — —). To 4.00 ml of 5.0×10^{-5} M ferrihaemoglobin in 0.05 M sodium phosphate (1:1) buffer (pH 6.84), 25 or 26 μ l of 0.1 M potassium ferricyanide and 12 μ l of 0.05 M arylhydrazine hydrochloride were added in succession. Spectra were recorded 1, 5, and 10 min after the addition of arylhydrazine. All three recordings are shown for 2,6-dichlorophenylhydrazine. Reactions with the other isomers were nearly complete after 1 min, and only the 5-min spectra are shown. The 10-min spectra showed little change. The spectra resulting from the 2,4-dichloro and 2,5-dichloro isomers were very similar but not identical to that resulting from the 2,3-dichloro isomer.



hydrazine¹²⁻¹⁴ can account for the marked difference in rate of haemolysis. Abnormally high concentrations of these reactive oxidants would lead to an accelerated rate of oxidation of the porphyrin rings^{15,16} and the thiol groups¹⁷ of phenylhydrazine-induced ferrihaemochrome. The resulting disruption of normal non-polar interactions between haem and globin and between the polypeptide chains of globin would then cause rapid formation of Heinz bodies, the presence of which is associated with haemolysis¹⁸. The induction of haemolytic anaemia only by those arylhydrazines that resulted in the quantitative formation of ferrihaemochromes favours a molecular mechanism for the induction of oxidative denaturation by an arylhydrazine in which the oxidation of susceptible groups within the haemoglobin molecule is facilitated by the destabilising effect of a large ligand that includes the aryl portion of the arylhydrazine.

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Role of hybrid tetramer formation in gelation of haemoglobin S

THE transformation of erythrocytes containing haemoglobin (Hb) S from pliable biconcave disks into rigid pointed "sickled" forms, which underlies the pathology of sickle cell disease, results from the tendency of this mutant haemoglobin to polymerise and produce an intracellular gel on deoxygenation. In the genetic variants of sickle cell disease, the different types and proportions of non-S haemoglobins present in the red cells together with Hb S are important in determining the extent of the sickling tendency and the severity of the disease. Red cells containing Hb S along with a substantial proportion of foetal haemoglobin (Hb F, $\alpha_2\gamma_2$), sickle less readily than cells having a similar proportion of the normal adult type, Hb A ($\alpha_2\beta_2$). Such differences can be demonstrated with solutions of haemoglobin mixtures *in vitro* by measuring the minimum gelling concentrations (MGC), that is, the minimum concentration of total haemoglobin at which the mixture will form a gel on complete deoxygenation. The higher MGC of Hb S-F mixtures compared with S-A mixtures correlates with the lesser sickling tendency of the former combination within red cells.

There is growing evidence that tetramers of deoxy Hb S ($\alpha_2\beta_2$) aggregate as helical polymers in sickled cells and gels, but it is not known whether non-S haemoglobins can be included in these polymers, or if they play some other part in gel formation. Ultracentrifugation of deoxygenated haemoglobin mixtures indicated that Hb F was excluded from the solid phase of the gel, whereas some Hb A was included¹. In solutions of oxyhaemoglobin, the tetramers are in a fairly

rapid dissociation equilibrium with $\alpha\beta$ dimers, and in mixtures of two haemoglobins (such as S-F or S-A) it has been shown that a large proportion of the haemoglobin exists as hybrid tetramers ($\alpha_2\beta^S\gamma$ or $\alpha_2\beta^A\beta^S$)². There have been little experimental data concerning the possible role of such hybrid tetramers in sickling and gelation. We have approached this problem by comparing the gelling behaviour of mixtures of haemoglobins in which hybrid formation was allowed to occur, or was prevented by two different methods.

The first method used to prevent hybrid formation was based on the extremely slow rate of dissociation of deoxyhaemoglobin tetramers. The non-S haemoglobin (Hb A or F) was deoxygenated with N₂ gas in one flask, while in another flask, oxygen was eliminated from a solution of Hb S or Hb C_{Harlem} ($\alpha_2\beta_2^{6Val,73Asn}$) which had been converted into the cyanmet form to prevent gelation. Although a solution once gelled could be liquified by chilling for the purpose of mixing, the non-gelling cyanmet form was chosen to avoid the possibility of partial aggregation which might prevent complete mixing. An appropriate volume of deoxyhaemoglobin A or F was then transferred anaerobically to the flask containing the oxygen-free cyanmet haemoglobin so that Hb S or C_{Harlem} comprised 40% of the final mixture. In spite of mixing, the deoxyhaemoglobin should remain almost entirely in the tetrameric state and thus be unable to hybridise. After chilling and thorough mixing, the cyanmet haemoglobin was converted to the deoxy form by anaerobic addition of sodium dithionite (one mol per mol total haem) and the MGC was determined at room temperature³. Controls for these experiments consisted of the same haemoglobins mixed in the oxy state to permit hybrid formation before deoxygenation and gelling.

The second method used to avoid hybridisation consisted of reacting the non-S haemoglobin component (Hb A or F) with a bifunctional cross-linking reagent, difluorodinitrophenylsulphone. Macleod and Hill have shown that the predominant reaction product contains an internal cross link between the amino termini of the two α chains⁴. After reaction, the cross-linked haemoglobin was separated from unreacted haemoglobin by gel filtration on Sephadex G-100 in 1 M MgCl₂; in this medium the non-cross-linked haemoglobin is fully dissociated into $\alpha\beta$ dimers whereas the cross-linked haemoglobin, fixed as tetramers, is eluted first by virtue of its higher molecular weight⁴. At the end of gelling experiments with mixtures of Hb S (or C_{Harlem}) and cross-linked haemoglobins A or F, electrofocusing in the deoxy state on polyacrylamide gels² confirmed the absence of hybrid tetramers. Control gelling experiments used the unreacted portion of the non-S haemoglobin eluted as the slower-moving fraction from the Sephadex column. After gelation, electrofocusing of these mixtures in the deoxy state revealed a large proportion of haemoglobin at the position of hybrid tetramers.

We studied three mixtures, Hb S + Hb A, Hb S + Hb F and Hb C_{Harlem} + Hb A. The effect of hybrid formation on gelation of mixtures of Hb A with C_{Harlem} was of special interest because of our earlier findings with this unusual mutant, in which each β chain carries both the "sickle substitution" β^{6Val} , and a second substitution, β^{73Asn} . Pure HbC_{Harlem} has a much higher MGC than Hb S, indicating that the β^{73Asn} substitution somehow inhibits gelling. Addition of Hb A, however, lowers the MGC of Hb C_{Harlem}, so that in mixtures containing 50% or more of Hb A, the MGC is equal to those of Hb S-A mixtures of equivalent proportions. Therefore, we postulated that the presence of hybrid tetramers having one normal β chain ($\alpha_2\beta^A\beta^{C_{Harlem}}$) might eliminate the inhibitory effect of β^{73Asn} (refs 5 and 6).

The results of the gelling experiments are shown in Table 1. As expected, with the usual gelling procedure (in which hybrids occur) the MGC is much higher with mixtures of Hb S and F than with S-A mixtures. By contrast, when hybrid formation was prevented by either technique, the gelling points of the S-F mixtures were much lower, and equal to those of S-A mixtures. Thus the inhibitory effect of Hb F on gelation (and

Table 1 Effect of hybrid tetramer formation on the MGC of haemoglobin mixtures

Hb composition of mixture (40:60)	Hybrids present		Hybrids absent (or minimal)	
	Hb mixed in oxy state	Hb A or F non-cross-linked control	Hb mixed in deoxy state	Hb A or F cross-linked
Hb S + Hb A	30.2	31.6	29.4	30.2
Hb S + Hb F	39.4	37.2	30.3	32.6
Hb C _H + Hb A	30.0	31.6	35.9	37.7

Haemoglobin dialysed against 0.15 M potassium phosphate, pH 7.35, deoxygenated with N₂ gas at 25 °C, sodium dithionite added, final pH of haemoglobin 7.1; mean MGC values expressed in g Hb dl⁻¹.

sickling) seems to require the formation of $\alpha_2\beta^S\gamma$ hybrids. These findings suggest that the presence of one γ chain in the tetramer is unfavourable for polymerisation. In Hb S-F mixtures, the formation of such hybrids lowers the number of Hb S tetramers which do polymerise well. Accordingly, if hybridisation is prevented, the gelling point is lower; in fact, the presence of non-hybridising Hb F tetramers seems to have an effect on gelation equal to that of Hb A.

With mixtures of Hb C_{Harlem} and Hb A, on the other hand, the effect of hybridisation was the opposite of that seen with the Hb S-F mixtures. When $\alpha_2\beta^A\beta^C$ hybrids were allowed to form, the MGC was equal to that of Hb S-A mixtures, whereas preventing formation of these hybrids led to a much higher gelling point. On the basis of earlier experiments, we suggested only one of the two β^{Val} regions of the Hb S tetramer provided an intermolecular contact in the polymer, while the other β chain probably provided other important contacts^{7,8}. The present findings support this idea, and suggest that the $\alpha_2\beta^A\beta^C$ hybrids enter the polymer. The normal β chains apparently favour polymerisation more than chains having a β^{3Asn} residue.

It was surprising to find that the presence or absence of hybridisation made little or no difference in the gelling of Hb S-A mixtures. Our findings differ from those of Moffat⁹, who reported slightly lower MGC values for Hb S and Hb A mixed in the oxy state than in the deoxy state. It is not clear whether this discrepancy stems from differences in experimental conditions (type of buffer, pH) or technique. One possible interpretation of our results is that, with Hb S-A mixtures, two effects of hybrid formation on gelation are counterbalanced; if we suppose that Hb S tetramers favour gelation more than SA hybrids, then when hybrid formation occurs, the effect of reducing the number of S tetramers may be balanced by the effect of increasing the total proportion of tetramers capable of polymerising (the remaining S tetramers and the SA hybrids).

The present data shed some additional light on the so-called "sparing" effect of Hb A on gelation of Hb S. Singer and Singer first pointed out that the addition of Hb A to Hb S reduced the partial concentration of Hb S required for gelation¹⁰. This is also found under the conditions of our experiments. The MGC of Hb S alone is about 22 g dl⁻¹ while that for a 40:60 mixture of Hb S with Hb A is about 30 g dl⁻¹, so that the partial concentration of Hb S is about 12 g dl⁻¹. Our experiments demonstrate that this sparing effect of Hb A does not require the formation of $\alpha_2\beta^A\beta^S$ hybrid tetramers. Furthermore if (and only if) hybrid formation is prevented, the same sparing effect is provided by Hb F. It seems unlikely that $\alpha_2\gamma_2$ tetramers would enter the polymer more readily than $\alpha_2\beta^S\gamma$ hybrids, the formation of which inhibits gelation. Thus, non-S Hb tetramers (A or F) seem to facilitate gelling in an equal, nonspecific manner which may not involve entry into the polymers.

These results may also help interpret our earlier findings that deoxygenated mixtures of Hb S with cyanmet Hb F showed the same gelling behaviour as Hb S-A mixtures³. Using

techniques of electrofocusing in the deoxy state, Bunn and McDonough have recently shown that cyanmethaemoglobin fails to form stable hybrids with deoxyhaemoglobin¹¹. Deoxygenation may promote dissociation of hybrids in a mixture of Hb S with cyanmet-Hb F, so that such a mixture may be comparable with the S-F mixtures in the present experiments in which hybrid formation was prevented. In that case, cyanmet Hb F or Hb A tetramers seem to facilitate gelling in mixtures with deoxy Hb S to the same extent as do deoxy-Hb A or F tetramers. As noted above, these effects need not involve entry into the helical polymers.

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Primary electron acceptor complex of photosystem I in spinach chloroplasts

PHOTOSYSTEM I (PSI) is the photochemical reaction complex involved in the generation of a low potential reductant in oxygen-evolving photosynthetic organisms. The primary photochemical reaction in PSI has been identified as the photo-oxidation of a reaction centre chlorophyll complex P700 (ref. 1). This photo-oxidation occurs both at room temperature and in the frozen state at temperatures as low as 4.2 K. At cryogenic temperatures this photo-oxidation has generally been found to be irreversible². Malkin and Bearden³ showed that the electron acceptor for this photo-oxidation reaction is a bound ferredoxin. This ferredoxin has two iron-sulphur centres with very low redox potentials ($E_{m10} = -550$ and -590 mV)⁴⁻⁶. It has been proposed that these centres are the primary electron acceptor of PSI^{3,4}. More recent evidence suggests it may not be⁷⁻¹¹, and we have shown¹¹ that when the bound ferredoxin is chemically reduced, illumination at low temperature results in the photo-oxidation of P700 without any change in the bound ferredoxin, this oxidation is reversible. In photosynthetic bacteria a component with an EPR signal at $g = 1.82$ has been identified as the primary electron acceptor of the photochemical system^{12,13} and it is possible that a similar component might be involved in chloroplast photochemical reactions. McIntosh *et al.*¹⁴ obtained kinetic evidence for the presence in PSI particles of a component with an EPR signal at $g = 2.06$ and $g = 1.76$ which showed a reversible redox change on illumination of the particles at low temperature parallel to that of P700. The changes observed were small and it was not possible to obtain a spectrum of the component. Using a more highly purified PSI preparation we have now obtained strong evidence that this component is the primary electron acceptor of PSI.

Figure 1 shows the EPR spectrum of a sample of PSI

particles frozen under illumination in the presence of sodium dithionite and methyl viologen. Spectrum A shows the spectrum at 18 K and 20 mW power. In these conditions the iron-sulphur centres can be clearly seen and are completely reduced. Spectrum B shows the spectrum of the same sample at 9 K and 100 mW. In these conditions the signals due to the iron-sulphur centres of the bound ferredoxin and the methyl viologen free radical show power saturation effects. A new signal can be seen at $g = 1.76$ and $g = 1.86$. This signal is frozen in only in samples prepared in the extreme reducing conditions used for this sample. For example, little or none of this signal can be seen in samples frozen with dithionite but without methyl viologen and is not observed in samples frozen with dithionite in the dark. It is observed in photosystem I particles, and seems to be most concentrated in those samples with the highest P700 to chlorophyll ratio. We have therefore concluded that it is an electron transport carrier with an extremely low redox potential associated with PSI.

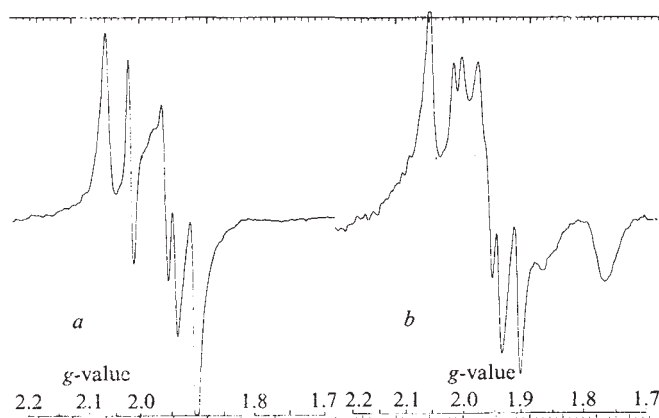


Fig. 1 EPR spectra of PSI particles showing the signal at $g = 1.76$ due to the primary electron acceptor of PSI. Highly purified particles were prepared from broken spinach chloroplasts¹⁵ by Triton treatment¹⁶ followed by DEAE chromatography. The sample was reduced by illumination at room temperature in the presence of 0.2% $\text{Na}_2\text{S}_2\text{O}_4$ and 15 μM methyl viologen. Chlorophyll concentration 1.5 mg ml^{-1} . EPR spectra were recorded as described previously^{4,11}. The conditions for each spectrum were as follows. *a*, Frequency 9.24 GHz, power 20 mW, modulation amplitude 10 G, scan rate 500 gauss min^{-1} , gain 500—temperature, 18 K. *b*, as for *a*, except that the temperature was 9 K and power 100 mW. The spectra were recorded in the dark.

The g value of this component is very similar to that of the kinetic change reported by McIntosh, *et al.*¹³. We have examined the effect of illumination on this signal. We have shown that in PSI particles frozen with both P700 and the bound ferredoxin in the reduced state the photo-oxidation of P700 is reversible¹¹. Figure 2 shows this reversible photo-oxidation in triton PSI particles. Figure 3 shows the effect of illumination of the same sample at lower temperature (9 K) and high power. The signal at $g = 2.00$ due to P700 is, much smaller in these conditions, however it can be seen that the signal at $g = 1.76$ is induced by illumination in these conditions and disappears again when the light is turned off. The kinetics of the time course shown in Figs 2 and 3 are machine limited. They do, however, indicate that P700 photo-oxidation and the appearance of the $g = 1.76$ signal occur on illumination and are fully reversible in the dark. The kinetic measurements of McIntosh *et al.* indicate that the decay kinetics of the two signals are the same. In samples in which the bound ferredoxin is oxidised before illumination, illumination in the conditions described here results in irreversible oxidation of P700 and reduction of the bound ferredoxin, little or no $g = 1.76$ signal is then observed. If the $g = 1.76$ component is frozen largely in the reduced state as in the

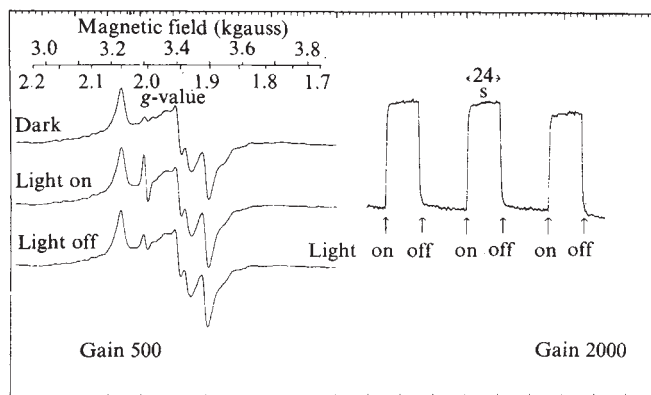


Fig. 2 The effect of illumination at low temperature on the EPR signal of P700 in Triton PSI particles. Triton PSI particles (1.5 mg chlorophyll ml^{-1}) were reduced by exposure to 0.2% $\text{Na}_2\text{S}_2\text{O}_4$ pH 9.0 for 45 min (ref. 11), before freezing to reduce the bound ferredoxins. Left, EPR spectrum in the $g = 2.00$ region. Conditions as in Fig. 1. Power 20 mW, temperature 18 K. The sample was illuminated in the spectrometer with a 1000 W projector. Right, time course of the effect of illumination on the P700 signal. Instrument settings as above except that the change in amplitude of the $g = 2.00$ signal was followed with time.

sample shown in Fig. 1 the extent of P700 photo-oxidation on illumination at cryogenic temperatures is greatly decreased. In digitonin-triton particles the $g = 1.76$ component is more enriched than in Triton particles and a signal at $g = 1.86$ is seen as well as at $g = 1.76$ on illumination. This observation together with that of McIntosh *et al.*¹⁴ who observed kinetic changes around $g = 2.06$ suggest that the component has an EPR spectrum around $g = 2.00$ with g_z about 2.06, g_y about 1.86 and g_x about 1.76. This spectrum might be due to an iron-sulphur centre in a novel environment or to an unknown type of iron centre as has been proposed for the bacterial primary electron acceptor.

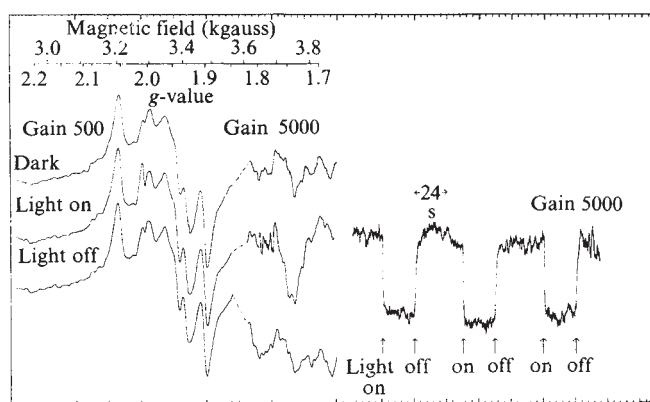


Fig. 3 The effect of illumination at low temperature on the $g = 1.76$ signal in PSI particles. The same sample was used as in Fig. 2. Left, EPR spectra in the $g = 2.00$ region. Instrument settings as in Fig. 1 with Power 100 mW, temperature 10 K. Gain as indicated. Right, time course of the effect of illumination on the $g = 1.76$ signal. Instrument settings as above except that the change in amplitude of the $g = 1.76$ signal was followed with time.

These experiments show that chloroplasts contain a redox carrier which is reduced, probably at very low potentials, in parallel with the photo-oxidation of P700. This reduction is reversible at temperatures down to 8 K. When this component is reduced P700 photo-oxidation is inhibited. When secondary electron acceptors are available P700 photo-oxidation is irreversible and this component cannot be detected. These

results suggest that the component with an EPR signal at $g = 1.76$ is the primary electron acceptor of PSI.

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Genetic relationship of a primate RNA tumour virus genome to genes in normal mice

HUEBNER and Todaro¹ and Temin² proposed that the genomes of RNA tumour viruses originate from genes in DNA of normal cells. Subsequently, a class of RNA tumour viruses (endogenous or class 1 viruses) was discovered and shown by molecular hybridisation to contain RNA genetically related or identical to genes in normal cells³⁻⁸. Both the biological and hybridisation data leave little doubt that normal cells carry genes capable of giving rise to class 1 RNA tumour viruses (see refs 7 and 8 for reviews). A second class of RNA tumour viruses is more distantly related to cell genes (exogenous or class 2 viruses, see refs 7 and 8). Mouse and chicken class 2 viruses, however, are genetically related to class 1 viruses from the same species⁷⁻¹² and the genome of one class 2 mouse RNA tumour virus (Rauscher leukaemia virus) consists primarily of RNA sequences that are related distantly to genes in normal mice but not to genes found in several other animals¹³. Indirect evidence has indicated that primate RNA tumour viruses and certain mouse RNA tumour viruses are unusually closely related^{10,11,14,15}, suggesting that the viruses have a common origin, for example, in genes of mice. We report here that a primate RNA tumour virus isolated originally from a woolly monkey sarcoma and passaged in marmosets does contain a genome that by molecular hybridisation criteria is related to genes found in mice. The data further indicate that a small portion of the primate RNA tumour virus genome is related to genes of normal primates.

The virus analysed here, simian sarcoma virus (SiSV) or woolly monkey virus, was isolated from a natural sarcoma

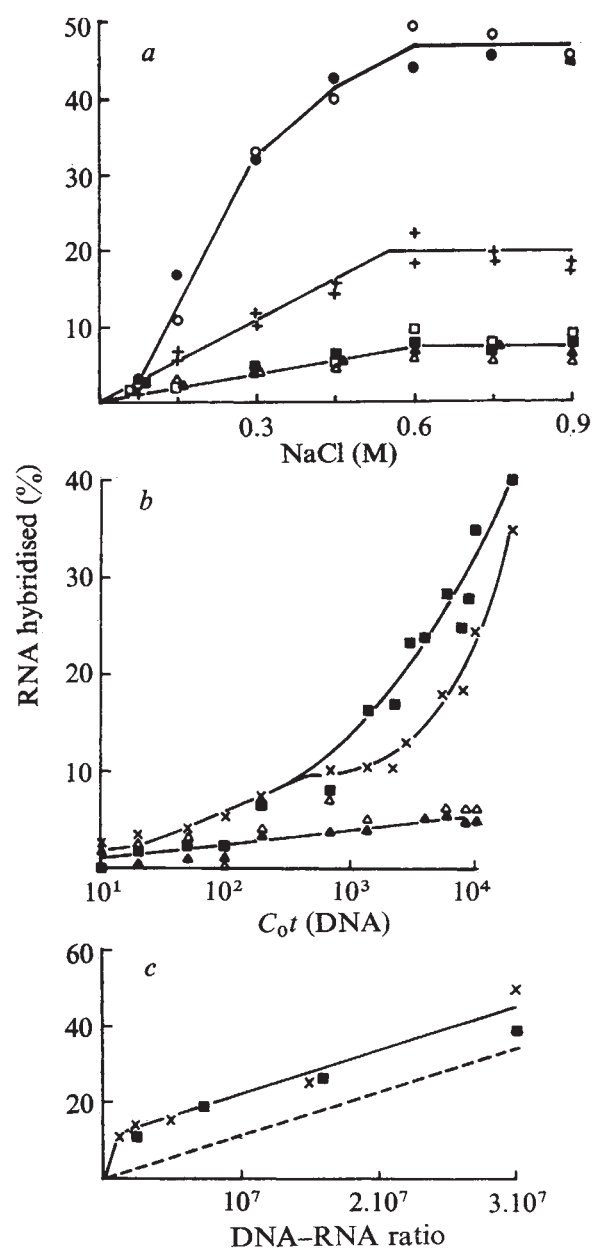


Fig. 1 Hybridisation of ¹²⁵I-RNA from SiSV to cell DNA. Hybridisation mixtures contained viral RNA and cell DNA (weight ratio = 1:8 × 10⁶, DNA concentration = 8.3 mg ml⁻¹, RNA = 500 c.p.m., 10⁸ c.p.m. μg⁻¹) in 0.4 M PO₄, pH 6.8. Mixtures were boiled for 5 min, then incubated at 60 °C. a, Hybridisation mixtures (5 μl) were incubated for 100 h (C₀t (DNA) = 10⁴), then exposed to 20 μg ml⁻¹ RNase at 37 °C for 120 min in the indicated concentration of NaCl. Acid-precipitable radioactivity was used to measure the percentage RNA hybridised^{4,13}. b, Hybridisation mixtures were incubated for different lengths of time, then exposed for 60 min at 37 °C to 20 μg ml⁻¹ RNase in 0.6 M NaCl. c, Hybridisation mixtures containing different DNA-RNA ratios (DNA = 10 mg ml⁻¹) were incubated for 160 h (C₀t (DNA) = 2 × 10⁴) then exposed for 60 min at 37 °C to 20 μg ml⁻¹ RNase in 0.6 M NaCl. Dashed line indicates hybrid yield as a function of the ratio DNA-RNA, subtracting the contribution by rapidly-annealing DNA sequences. This function was subjected to a double reciprocal analysis (1/hybrid yield against RNA-DNA ratio) and yielded an x-intercept value of 0.022 (45% RNA hybridised). This is taken to reflect the percentage RNA that could be hybridised to infrequent DNA sequences at infinite DNA input. Percentage RNA that could complex repeated plus infrequent DNA sequences at infinite DNA input is therefore 56. Sources of cell DNA: a: ○, T1API (SiSV); NRK (SiSV); +, mouse; □, gibbon; ■, woolly monkey; ▲, chimp; △, human; b and c: ■, HF (SiSV); ×, mouse; △, woolly monkey; ▲, human. Less than 25% of the acid-precipitable radioactivity was lost from the RNA after 160 h of incubation at 60 °C. RNase-resistant radioactivity obtained without DNA are subtracted from each hybrid yield value (3-5% of the input radioactivity).

of a woolly monkey¹⁶. The monkey tumour was homogenised, and a cell-free extract was placed on normal marmoset lung cells (line 1283), which then began to produce virus. The virus-producing cells were inoculated into a marmoset (71AP1) causing the development of a tumour which was explanted and grown in culture. The tumour cells produced a virus, referred to here as SiSV (71AP1). To the best of available knowledge, none of the biological materials, save possibly the original woolly monkey itself, had direct contact with rodents or rodent cells. SiSV (71AP1) has subsequently been introduced into several other cell lines, including marmoset HF cells and normal rat kidney NRK cells.

The experiments reported here involve hybridisation of 70S RNA isolated from SiSV (71AP1) and labelled with ¹²⁵I *in vitro*¹⁷ to DNA purified from a variety of animal tissues or cultured cell lines. As the objective was to examine genes of normal cells for sequences related but not necessarily identical to the viral RNA, conditions of hybrid formation and detection capable of monitoring imperfect RNA-DNA complexes was required. Hybridisation of viral RNA to cell DNA at 60 °C in 0.4 M PO₄ has been found to be a suitable condition of hybrid formation for this purpose¹³. The condition of hybrid detection most suitable for detecting imperfect complexes formed between SiSV (71AP1) RNA and cell DNA (8×10⁶-fold weight excess) was treatment of the hybridisation mixture with 20 µg ml⁻¹ RNase A in 0.6 M NaCl (Fig. 1a). The experiments used a 120 min exposure of the hybrid mixture to RNase. In these conditions, RNA from SiSV (71AP1) formed RNase-resistant complexes with DNA from rat NRK or marmoset 71AP1 cells infected with SiSV (50% of the RNA) and with DNA from normal mice (20% of the RNA). The RNA-DNA hybrids were maximally protected from degradation by RNase in 0.6 M NaCl; some complexes were destroyed in solutions containing less NaCl. Hybrid yield using DNA from uninfected primates was only 5–10% of the RNA when RNase treatment was carried out for 120 min (Fig. 1a), but was as high as 20–25% when RNase treatment was limited to 60 min (Table 1).

Table 1 presents hybridisation results for ¹²⁵I RNA from SiSV (71AP1) to DNA from many animals, using a single condition of hybrid formation (60 °C, 0.4 M PO₄, C₀t=10⁴) and hybrid detection (RNase treatment in 0.6 M NaCl at 37 °C for 60 min). DNA from mice or rats hybridised more SiSV RNA than did DNA from other animals, but appreciable hybridisation was obtained with DNA from some Old World primates and, to a lesser extent, from New World monkeys. DNA from chimpanzees hybridised 25% of the SiSV RNA, whereas DNA from humans hybridised only 5–10%. DNA from the lower animals examined, excluding mice and rats, hybridised only 1–10% of the RNA.

The kinetics of hybridisation of SiSV (71AP1) RNA to DNA from NIH Swiss mice or from SiSV-infected marmoset HF cells was that expected from DNA sequences present only a small number of times per genome (Fig. 1b). By a C₀t of 2×10⁴, 35% of the RNA had hybridised to DNA from mice and there was no indication that the hybridisation reaction was slowing. A small portion of the RNA (10%) annealed rapidly to DNA from mice or infected marmoset HF cells. DNA from humans or woolly monkeys reacted poorly with SiSV RNA, even at high C₀t. The t_m in 0.15 M NaCl of the hybrid structures formed with DNA from mice or chimpanzees was only about 65 °C, compared with 80 °C for hybrids involving DNA from SiSV-infected marmoset 71AP1 cells. Therefore, the virus-related sequences in the DNA of mice and chimpanzees are less closely related to the viral genome than are the proviral DNA sequences in the DNA of SiSV-infected cells.

The DNA-RNA ratio in the experiments described above was 8×10⁶. If each viral subunit has a molecular weight of

Table 1 Hybridisation of RNA from SiSV to DNA from various animals

DNA source	Hybrid yield (% RNA hybridised)
Mice, rats and infected cells	
71AP1 (SiSV) (C)	55
NIH Swiss mouse (S, L)	25
A31 mouse (C)	23
Wild-type mouse (S, L)	15
MOPC mouse (Tu)	22
NRK normal rat kidney (C)	16
Old World primates	
Chimpanzee (M)	25
Gibbon (B)	20
Celebes ape (M)	21
Hamadrayas baboon (T)	15
Mandrill baboon (B)	16
Slow loris (M)	15
Griquet (M)	6
Vervet (T)	9
Crab-eating macaque	7
Stumptail macaque	8
Human (WBC)	5
New World primates	
Capuchin (T)	18
Marmoset (L)	12
Owl monkey (T)	11
Spider monkey (M)	11
Howler monkey (M)	11
Woolly monkey (S)	11
Squirrel monkey (S)	7
Other animals	
Pig (L)	10
Squirrel (S, L)	8
Cat (S, L)	8
Chicken (E)	4
Raccoon (S, L)	2
Slug (A)	1

RNA-DNA hybridisation mixtures were constructed as described in the legend to Fig. 1a, then exposed to 20 µg ml⁻¹ RNase at 37 °C for 60 min in 0.6 M NaCl. Letters in parentheses indicate the tissue or cell type used to obtain DNA. C, Cultured cells; S, spleen; L, liver; Tu, tumour; M, muscle; B, brain; T, thymus; E, embryo; A, whole animal; WBC, White blood cells.

3×10⁶ (ref. 18) and if the viral-related sequences exist only once per genome, complementary DNA should be 27-fold in excess. If the viral-related sequences in the mouse DNA, however, are only distantly related to RNA from SiSV (71AP1), after hybridisation the RNA-DNA hybrids can exist in reversible equilibrium with free RNA. Raising the amount of DNA would then drive more RNA into RNA-DNA hybrids. Figure 1c shows that at a C₀t of 2×10⁴, increasing the DNA-RNA ratio to 30×10⁶, resulted in hybridisation of 45% of the SiSV (71AP1) RNA with normal mouse DNA—a hybrid yield equivalent to that obtained with DNA from SiSV-infected marmoset HF cells. Again there was indication that 10–11% of the RNA hybridised to repeated DNA sequences. These data when analysed by a double reciprocal representation showed that 56% of the SiSV RNA would have hybridised to mouse DNA at infinite DNA input (see legend to Fig. 1).

Several reports¹⁴ have suggested an unusual genetic proximity between viral sequences in primates, including man, and those in viruses from mice^{10,11,13}. It has also been demonstrated that both 'endogenous' and 'exogenous' mouse RNA tumour viruses carry genomes related to DNA sequences in normal mice^{7,13,19}. Taken together, these observations indicate that the primate viruses also contain genomes related to genes in normal mice, a conclusion verified by our studies. The concept of mouse-primate relationship of RNA tumour virus nucleotide sequences is supported by the isolation from cultured asian mouse cells of a virus containing proteins antigenically related to analogous proteins from primate RNA tumour viruses²⁰.

The homology between SiSV RNA and normal mouse

DNA can be used as an indication that SiSV originated in mice and subsequently entered primates. The weaker homology between SiSV RNA and primate DNA may reflect genetic change promoted by recombination with the new host². The hybridisation results show a closer homology of the virus to Old World monkeys than to New World monkeys, indicating genetic interaction of the virus with Old World monkeys. SiSV (71AP1) was isolated from and has subsequently had contact solely with New World monkeys or cells derived from New World monkeys. We have been told by the owner of the woolly monkey from which SiSV was obtained that this household pet cohabited for 1-2 years with a gibbon before the woolly monkey developed a sarcoma. Possibly, the gibbon carried the virus and transmitted it to the woolly monkey. The possibility of an Old World primate transmitter of a mouse virus as a means for generating primate RNA tumour viruses is being studied.

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Specific binding of CAP factor to *lac* promoter DNA

CATABOLITE repression in *Escherichia coli* is mediated by the cyclic AMP dependent catabolite activator protein (CAP). Growth conditions where the induced catabolism of certain sugars is energetically favourable, primarily in the absence of glucose, promote the intracellular induction of a high cyclic AMP concentration; CAP binds cyclic AMP, is activated, and stimulates mRNA synthesis from promoters of catabolite repressible operons¹⁻⁴. The mechanism by which CAP influences the interaction between RNA polymerase and promoter DNA is unknown. Purified CAP preparations exhibit a cyclic AMP-dependent DNA binding activity, but efforts to detect specific binding to promoters of catabolite repressible genes have been unsuccessful; all DNAs tested have shown similar binding affinities^{5,6}. The only evidence for a specific interaction between CAP and promoter DNA is a fluorescent probe study which showed that a *lac* transducing phage DNA molecule induces a conformational change in CAP that is not seen with the wild-type phage molecule⁷; but no

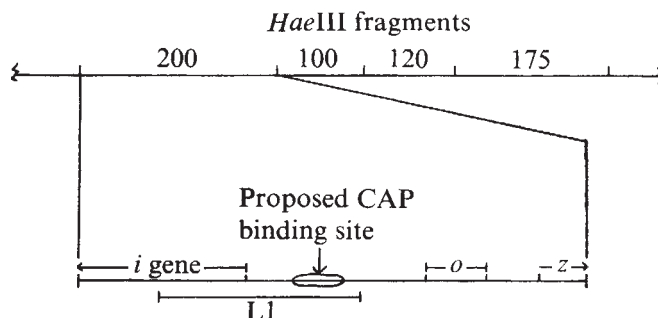
effort was made to correlate this difference with *in vivo* CAP behaviour. Here I report experiments which demonstrate that the CAP factor binds specifically to a site within the *lac* promoter. This binding is sensitive to mutations which affect the ability of CAP to stimulate *in vivo* and *in vitro* *lac* gene expression.

These experiments were made possible by the recent isolation of a 200 base pair DNA fragment containing an intact *lac* control region⁸. The fragment, designated *Hae*200, was generated by digesting 1,000 base pair sonicated *lac* fragments with the *Hae*III restriction endonuclease of *Haemophilus aegyptius*⁹ and was shown to contain the entire control region by the following criteria: first, an *in vitro* transcription system using *Hae*200 as a template synthesises mRNA which extends into the region coding for the NH₂ terminus of β -galactosidase, the product of the *z* gene (J. M., unpublished). Second, polyacrylamide gel electrophoresis of a digest of DNA from a strain containing the L1 deletion, known to cover both the COOH-terminus of the *i* gene and part of the *lac* promoter^{10,11}, shows that only the mobility of the *Hae*200 fragment is altered by the deletion⁸. Therefore the entire region from the end of the *i* gene to the beginning of the *z* gene must be on the fragment. Figure 1 shows a map of the *lac* region and the fragments generated by *Hae*III digestion.

I have investigated the ability of CAP to bind these fragments to nitrocellulose filters; details of the binding conditions are given in the legend to Fig. 2. The CAP preparation used in these assays, when used in an *in vitro* transcription system, will stimulate mRNA synthesis off the *Hae*200 fragment up to twentyfold. This stimulation is dependent on cyclic AMP and saturates at CAP concentrations similar to the maximum concentrations used in the binding assays (J. M., unpublished). All binding was carried out in the presence of a large excess of unlabelled, sonicated λ plac₅ DNA fragments; in the absence of these fragments no specific binding could be detected. Figure 2 displays the DNA binding activity of CAP towards the *Hae*200, *Hae*175, and *Hae*120 fragments. In these conditions CAP shows significantly greater affinity for the promoter containing *Hae*200 fragment than for either of the other fragments. The binding is dependent on cyclic AMP and can be inhibited by cyclic GMP, a competitive inhibitor of cyclic AMP¹². At higher concentrations of CAP the specificity becomes considerably less dramatic as the effect reported here is dependent on both the absolute CAP concentration and the CAP-DNA ratio.

If the affinity for *Hae*200 is functional it should be affected by promoter mutations which alter the ability of CAP to stimulate *lac* transcription. A suitable test for this is the CAP independent double mutant L8-UV5. L8, probably a point mutation, maps under the L1 deletion and gives a fifteenfold reduction in CAP stimulation¹⁰⁻¹². UV5 is a second mutation which reverts L8 to a CAP independent, lac⁺ phenotype¹³. Figure 3 shows that the affinity of CAP for *Hae*200 from L8-UV5 is significantly less than that for the wild-type frag-

Fig. 1 Map of the *lac* control region with the location of *Hae*III cuts as determined by Gilbert et al.⁸. Regions of interest on *Hae*200 are given in more detail, as determined by Gilbert et al.¹⁶, Dickson et al.¹⁴ and Beckwith et al.¹³.



ment, especially at low CAP concentrations; although the *Hae*175 fragments from the same preparations of the two strains bind with equal affinity (see also Fig. 2). The L1 deletion itself completely eliminates the ability of CAP to influence *lac* transcription^{10,11}. Figure 3 shows that the fragment corresponding to *Hae*200 isolated from the L1 strain (about 80 base pairs shorter than that from wild type) also shows little affinity for CAP. Similar effects have been seen with CAP preparations

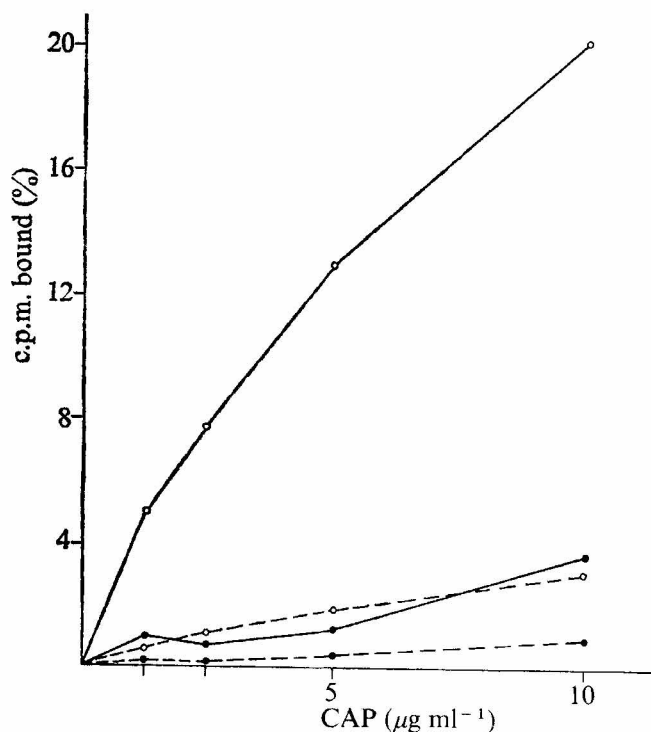


Fig. 2 Filter binding affinity of CAP for the *Hae*200, *Hae*175, and *Hae*120 fragments. Each binding reaction contains in 200 μ l: 20 mM Tris-HCl pH 8.0, 10 mM KCl, 3 mM MgCl₂, 0.1 mM EDTA, 0.25 mM cyclic AMP or cyclic GMP, 100 μ g ml⁻¹ bovine serum albumin, 10 μ g ml⁻¹ sonicated λ plac₈ fragments, and ³²P-labelled restriction fragments, from 1,000 to 5,000 c.p.m. per assay. Labelled fragments were purified as described by Gilbert and Maxam¹⁷, the specific activity of the fragments was about 10 Ci per mM phosphate. The fragments, dialysed into 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA, were made up to 10 mM KCl, 10 mM MgCl₂, 0.1 mM dithiothreitol and 200 μ g ml⁻¹ BSA. 10 μ g of carrier, sonicated 1,000 base pair fragments were added along with enough *Hae*III enzyme to yield a complete digest after an incubation of one hour at 37 °C. The digested fragments were made up to 2 M NH₄OAc and precipitated with two volumes of ice-cold 95% ethanol. They were resuspended in 0.5 M NH₄OAc, again ethanol precipitated, then electrophoresed through a 5% polyacrylamide TBM (0.1 M Tris-borate pH 8.3, 2 mM MgCl₂) gel¹⁷. The radioactive bands, identified by autoradiography, were cut out and eluted into 0.5 ml of 0.5 M NH₄OAc, 10 mM Mg(OAc)₂, 0.1 mM EDTA, 0.1% SDS, containing 20 μ g of tRNA. The DNA was then ethanol precipitated, resuspended in 0.5 M NH₄OAc, reprecipitated, and stored in 100 μ l of 10 mM Tris-HCl pH 7.5. CAP was purified by the method of Pastan *et al.*¹⁸, modified by the omission of dithiothreitol throughout. The purified protein was dialysed into 20 mM Tris-HCl pH 8.0, 50 mM KCl and stored at 4 °C. For the binding assay, salts, cyclic AMP, CAP, DNA fragments and BSA were incubated for 10 min at room temperature in silicated glass tubes, then filtered through S & S B-6 nitrocellulose filters that had been presoaked in 0.1 N NaOH, washed thoroughly in double distilled water and equilibrated with binding buffer. The filters were then washed twice with 0.15 ml binding buffer without BSA, dried thoroughly, and counted in toluene Omnifluor. Backgrounds in which no CAP was added have been subtracted in all cases. Typically these were between 5 and 10% of the input counts. Those cases in which bound counts were below background have been recorded as 0% counts bound. $\circ$ — $\circ$, Wild-type *Hae*200+cyclic AMP; $\bullet$ — $\bullet$, wild-type *Hae*200+cyclic GMP; $\circ$ — $\circ$ — $\circ$, wild-type *Hae*175+cyclic AMP; $\bullet$ — $\bullet$ — $\bullet$, wild-type *Hae*120+cyclic AMP.

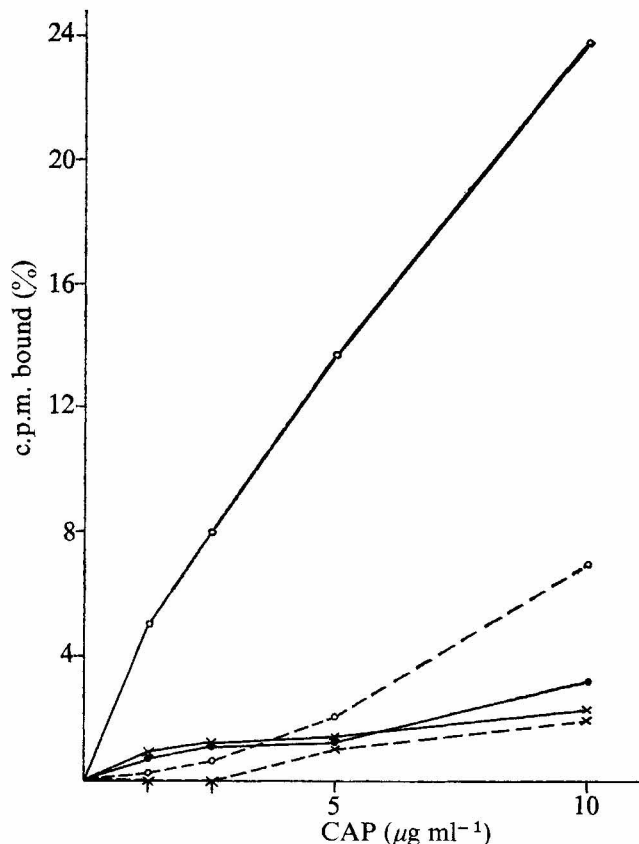


Fig. 3 *a*, Comparison of the binding of wild-type *Hae*200 to that of two mutants, L8-UV5 and L1. Conditions are the same as described in the legend to Fig. 2. 0.25 mM cyclic AMP was present in each assay. $\circ$ — $\circ$, Wild-type *Hae*200; $\circ$ — $\circ$ — $\circ$, L8-UV5 *Hae*200; $\bullet$ — $\bullet$, L8-UV5 *Hae*175; $\times$ — $\times$, L1 *Hae*200; $\times$ — $\times$ — $\times$, L1 *Hae*175.

purified in different ways and with several DNA preparations. I conclude that the observed DNA binding activity is functional and must be involved in the CAP regulatory function.

As for the mechanism of this regulation, the *lac* promoter sequence of Dickson *et al.*¹⁴ reveals that the L1 deletion covers a symmetry region of about 16 bases which may be the CAP binding site. This site is about 10 base pairs removed from a region to the right of the L1 deletion in which several mutations have been mapped and sequenced which reduce the affinity of the polymerase for the promoter in a *crp*⁻ background^{14,15}. The polymerase probably recognises the DNA sequence in this mutable region and this recognition event is a likely candidate for CAP regulation. This regulation may be effected either by a change in the conformation of the DNA, in which case there would be no direct CAP-polymerase interaction, or by a direct stabilisation through a CAP-polymerase-DNA complex. Although CAP has not been shown to interact directly with the RNA polymerase, conformational changes induced by promoter DNA sequence information may make possible such an interaction. Alternatively, the direct protein-protein interaction needed (about 2–3 kcalorie since CAP enhances the promotion by a factor of fiftyfold *in vivo*) may be too weak to have been detected by the methods used so far.

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Translation of plant messengers in egg cells of *Xenopus laevis*

MAMMALIAN and plant virus messengers can be translated in living egg cells and oocytes of the tadpole of *Xenopus laevis*¹⁻⁶, and this method could be useful in the study of messenger

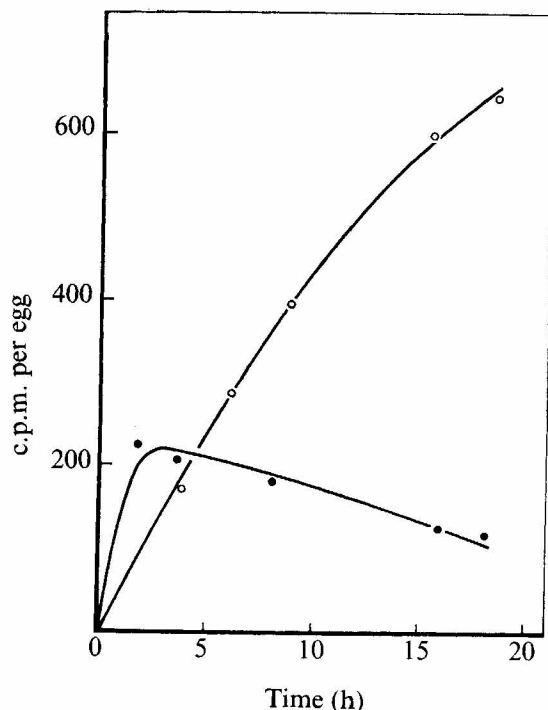


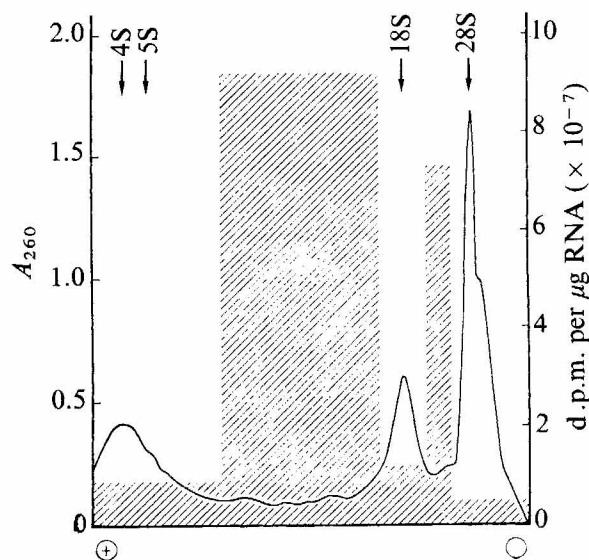
Fig. 1 The effect of injection of crude plant RNA extract on the synthesis acid precipitable material by egg cells of *Xenopus laevis*. The RNA was extracted from self-pollinated styles of *Petunia hybrida* using the cold phenol method⁷; 20-30 ng RNA was injected per egg together with 0.25 μ Ci ³H-leucine (specific activity 32 Ci mmol⁻¹) in a total volume of 100 nl injection medium⁴. In the control experiments the same amount of radioactivity was injected also in 100 nl. Injection was carried out with a microinjection device with which nanolitre quantities could be injected without precalibration¹¹. After incubation during the indicated times in saline solution¹ containing 1 mg actinomycin D l⁻¹, eggs were homogenised in 0.5 ml of a buffer containing 0.05 M Tris-HCl (pH, 7.6), 5 mM Mg acetate, 25 mM NH₄Cl, 6 mM β -mercaptoethanol and 0.2% SDS in a Braun homogeniser, and finally taken up in 2 ml of this buffer. After centrifugation at 2,500g at 4 °C an equal volume of 20% TCA was added to the supernatants and after 30 min at 0 °C the precipitate was centrifuged down at 30,000g. The pellet was resuspended in 10% TCA, heated at 90 °C for 15 min, cooled and again centrifuged at 30,000g. The pellet was resuspended in 0.1 ml of a proper buffer per egg cell; 10 μ l was counted in a toluene-based scintillation liquid in a Philips Liquid Scintillation Counter. ●, No RNA injected; ○, 20-30 ng total RNA extract injected per egg cell.

activity in general. Here I describe the results of experiments aimed at the use of this method for studying plant messenger RNA.

During the incompatibility reaction in *Petunia hybrida*, the style reacts to self and cross pollination by differential gene activity as measured by the synthesis of RNA⁷. Since these differences were most likely to be observed in messenger RNA, protein synthesis was also studied. Polysomes extracted from pollinated styles were incubated *in vitro* with a 105,000g rat liver supernatant⁸. The quantitative patterns of protein and RNA synthesis were very similar in the time after pollination⁹. Qualitative analysis of the newly synthesised polypeptides by means of electrophoresis, however, with or without SDS and urea, and isoelectric focusing, always showed one broad dominant peak and very little incorporation in other parts of the gel. These results did not agree with those obtained for RNA synthesis⁹. Nevertheless, this *in vitro* system demonstrated that it was possible to use animal enzymes to study the translation of plant messengers. It was therefore decided to investigate the possibility of using *Xenopus* egg cells for this purpose. Unfertilised egg cells could be collected the morning after injection of a female with hormone¹⁰. The egg cells were treated according to the procedures described by Gurdon *et al.*¹ except for the addition of 1 mg actinomycin D per l incubation medium. The effect of injection of total RNA extract from self-pollinated styles is given in Fig 1. At first the plant RNA tends to inhibit the translation of endogenous RNA but subsequently its translation occurs at an almost constant rate for several hours.

To prove that the level of incorporation is proportional to the amount of mRNA injected into the cell and depends exclusively on mRNA, a total cold phenol extract from five self-pollinated styles was electrophoretically separated in a tandem polyacrylamide gel⁶. The gel was then cut into five parts, comprising the RNA with sedimentation values of 28S and more, between 18S and 28S, 18S, between 5S and 18S, and 5S or less. Equal amounts of the re-extracted RNA were injected into *Xenopus* eggs and the radioactivity in the acid-precipitable material was measured (Fig. 2).

Fig. 2 The stimulation of incorporation of ³H-leucine by different RNA fractions. A crude RNA extract from 10 styles was separated electrophoretically in polyacrylamide tandem gels⁷. After electrophoresis the gels were cut into five parts. The corresponding parts were homogenised in such a volume of double concentrated injection medium that the concentration of RNA in all fractions was about 20 ng per 50 nl. The gel slurry was centrifuged at 10,000g for 10 min at 4 °C and the supernatants collected. Aliquots were diluted 1:1 with ³H-leucine and 100 nl of this mixture was injected into each egg cell. Incubation, extraction and measurement of radioactivity were as described in Fig. 1. Solid line; absorbance trace of one gel. Hatched blocks; Incorporation of ³H-leucine per μ g RNA.



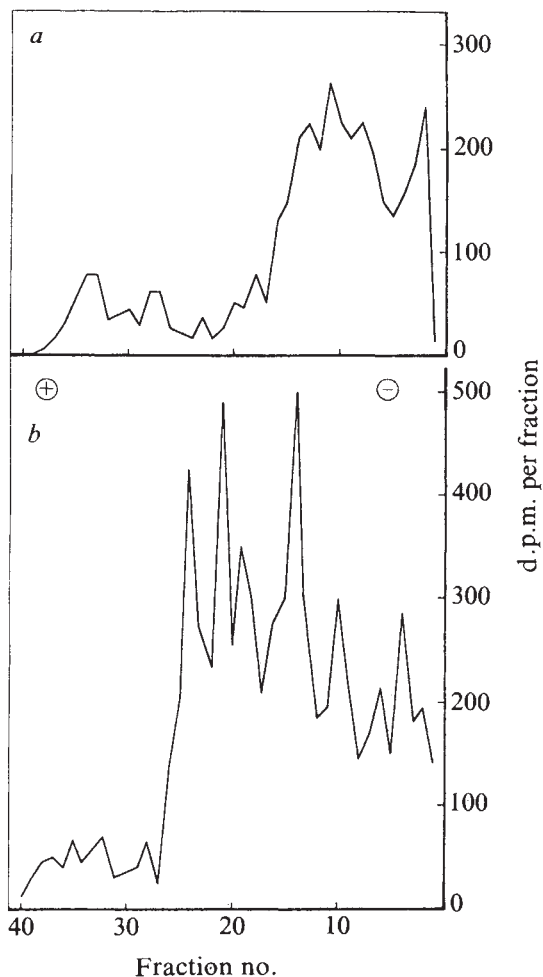


Fig. 3 Distribution of radioactivity after electrophoresis of protein extracts from treated *Xenopus* egg cells. Electrophoresis was carried out in the lower gel of 13.5% polyacrylamide with a stacking gel of 2.7% polyacrylamide at 3 mA per gel and 70 V in the presence of 4 M urea at pH 8.3 (0.05 M Tris-glycine buffer containing 0.2% SDS). Electrophoresis was stopped when cytochrome *c* had migrated 25 mm in the lower gel. The stacking gel was removed and counted as fraction number 1. The lower gel was cut into 1-mm slices (Mickle Gel Slicer). Gel slices were combusted (Packard Sample Oxidiser) and counted. *a*, Only endogenous RNA is translated; *b*, 25 ng of total RNA extract injected per egg cell.

Although most of the RNA was recovered as ribosomal and transfer RNA, the highest incorporation levels per mg RNA were obtained after injection of RNA from the parts of the gel in between those peaks. The conclusion, therefore, has to be that incorporation of ^3H -leucine only depends on the amount of messenger injected. The most difficult part of this study, however, is to prove that the proteins synthesised in *Xenopus* after injection of plant RNA contain plant proteins. After electrophoretic separation in SDS-urea-polyacrylamide gel the patterns of distribution of label were different for proteins synthesised in either the presence or absence of total plant RNA extract (Fig. 3). Immunological proof of identity will be conclusive when only one messenger is used and the protein synthesised this way has antigenic activity. When a complete RNA extract is used, however, the method becomes questionable, especially since many plant proteins have no antigenic activity. In this special case, however, RNA was extracted from self-pollinated self-incompatible styles. Thus it could be assumed that part of the proteins translated from it were involved in the incompatibility reaction, that is the inhibition of the pollen tube growth in the style. Therefore, if the right plant proteins were synthesised in *Xenopus*, application of these proteins to cross-pollinated styles would have the same effect on pollen tube length as a protein extract from self-pollinated styles, whereas

Table 1 The effect of proteins synthesised in *Xenopus* eggs on pollen tube length

Pollination	Addition	% Reduction of compatible tube length
Compatible ($S_3 \times S_2$)	—	0
	Water/buffer	2
	Endogenous <i>Xenopus</i> proteins	2–5
	Protein extract of <i>Xenopus</i> after injection of RNA from $S_3 \times S_3$	30–40
Incompatible ($S_3 \times S_3$)	Protein extract of self-pollinated styles	20–40
	—	20–40

Protein samples were obtained as described in Fig. 1, except for heating at 90 °C, but dialysed against running tap water, double distilled water or electrophoresis buffer (see Fig. 3) for at least 24 h. They were subsequently applied to the style by dipping the stigma in this solution, containing 400–600 µg protein per ml as determined according to Lowry *et al.* After 30 min at room temperature the styles were pollinated as indicated and incubated at 25 °C for 24 h. Pollen tube lengths were measured by ultraviolet fluorescence microscopy¹².

proteins synthesised with endogenous *Xenopus* RNA would have no effect. The results are as listed in Table 1.

Proteins synthesised in *Xenopus* after injection of RNA extracted from cross-pollinated styles also showed an effect on pollen tube growth. But stimulation was found only when the proteins were tested in a combination of pollen and style that did not contain any of the *S* alleles present in the combination of pollen and style from which the RNA was extracted. Reduction of pollen tube length was found when these *S* alleles were identical. These results indicate that the primary gene product of the *S* allele is synthesised in the style after pollination. They also tend to show that the polypeptides are involved in the recognition reaction which is very specific. Since these polypeptides can be synthesised in *Xenopus* egg cells with plant messengers, we may conclude that the machinery for the synthesis of proteins in eukaryotic plants and animals is very similar and that this method provides a useful tool in the study of gene activity in both plants and animals.

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Aminoacylation of transfer RNA microinjected into *Xenopus laevis* oocytes

THE microinjection technique perfected by Gurdon and his collaborators^{1,2} represents an excellent method for the study of the *in vivo* effects of drastically changing the molecular contents of the injected cells. We have demonstrated³ that radioactive yeast transfer RNA injected into oocytes of *Xenopus laevis* is not appreciably degraded after 20 h of

incubation inside these cells. It seems therefore, possible to modify the quantity and characteristics of the tRNAs present in frog oocytes and to test the effects that such changes may have on protein synthesis and other cellular processes. An important control in such a study is to determine whether the tRNAs microinjected into frog oocytes are functional in the sense that they can be aminoacylated by the recipient cell. This report presents evidence demonstrating that the amphibian oocyte is capable of aminoacylating yeast tRNAs injected into these cells at concentrations which are greatly in excess of their endogenous tRNA content.

The method used for microinjection of oocytes³ and for the preparation of protein synthesis elongation factor 1 from wheat embryos⁴ have been published previously. Specific

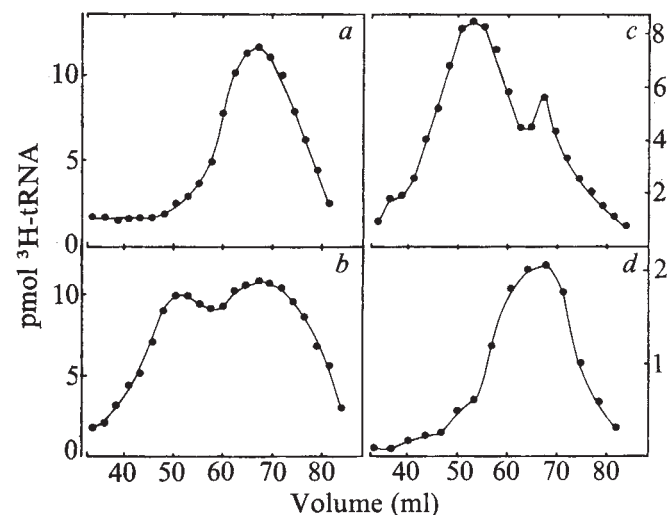


Fig. 1 The aminoacylation of yeast ^3H -tRNA injected into *Xenopus* oocytes assayed by the formation of ternary complex with EF1 and GTP. Bulk yeast ^3H -tRNA was prepared by the method of Brungaber¹² from a uracil-requiring strain of yeast (13088) grown in ^3H -uracil. This method includes a step to deacylate endogenous aminoacyl-tRNAs. Carrier yeast tRNA was added to facilitate isolation. The specific activity of this material was of 500 c.p.m. pmol^{-1} . Electrophoretic analysis showed that this material contained negligible 5S RNA. *a*, 80,000 c.p.m. of the uninjected ^3H -tRNA was incubated with 2 mg of wheat EF1 and GTP as described by Tarragó *et al.*¹³ and passed through a Sephadex G-200 column (55×1.5 cm) which was equilibrated and eluted with a buffer containing 20 mM Tris-HCl pH 7.5, 10 mM MgCl_2 , 50 mM NH_4Cl , 1 mM GSH, and 75 μmol GTP. Fractions of 1 ml were collected and aliquots were precipitated with cold 5% TCA, filtered through Millipore membranes and counted. *b*, 54,000 c.p.m. of ^3H -tRNA (specific activity 140 c.p.m. pmol^{-1}) was incubated and passed through the same column in identical conditions. This ^3H -tRNA has been aminoacylated *in vitro* using a crude wheat embryo aminoacyl-tRNA synthetase solution prepared according to Muench and Berg¹⁴ and a mixture of the 20 natural non-radioactive amino acids. A control experiment showed that the reaction mixture could aminoacylate 1.5% of yeast tRNA with phenylalanine. *c*, Yeast ^3H -tRNA recovered after its injection into *Xenopus laevis* oocytes was fractionated in the presence of EF1 and GTP as in *a*. This material containing a total of 120,000 c.p.m. with a specific activity 500 c.p.m. pmol^{-1} , had been injected into 50 *Xenopus laevis* oocytes (50 nl per oocyte) and these cells were incubated at 21 °C for 5 h in amphibian saline. The ^3H -tRNA was recovered by phenol extraction as described in Table 1 and the water phase was precipitated with 3 volumes of ethanol at -20 °C. The precipitate was collected by centrifugation at 20,000 g for 30 min and the ^3H -tRNA was redissolved in 200 μl of water and added to the ternary complex incubation mixture. *d*, Fractions 40 to 56 of *c* were pooled and precipitated with ethanol as in *c* and the ^3H -tRNA was dissolved in 0.5 ml of H_2O and brought to pH 10 with NH_4OH and incubated at 37 °C for 30 min. This treatment deacylates the tRNA and inactivates the wheat EF1 that might be present in these fractions. The mixture was then neutralised with HCl and reprecipitated with ethanol. The precipitate was dissolved in 200 μl of H_2O and incubated with EF1 and GTP as in *a*. 27,000 c.p.m. of ^3H -tRNA were run on this column.

details of the experimental procedures are given in the legends to the figures and table.

Controls on the state of the ^3H -tRNA recovered after microinjection and on the efficiency of recovery were performed. Electrophoresis on 7.5% polyacrylamide gels according to Peacock and Dingman⁵ showed that the material recovered by phenol extraction of oocytes 5 h after microinjection had mobility identical to the original ^3H -yeast tRNA. Microinjection of ^{14}C -Phe-tRNA followed immediately by phenol extraction of the injected cells gave a recovery of approximately 80% of the acid precipitable radioactivity in the water phase.

The small amounts of tRNA that could be injected and recovered from these cells and the necessity to differentiate the injected material from the endogenous tRNA required a new method for the assay of the aminoacylation of total yeast ^3H -tRNA. The method of choice determines the capacity of the ^3H -tRNA recovered from the injected cells to form a ternary complex with GTP and with protein synthesis elongation factor 1 (EF1) from wheat embryos. It has been demonstrated previously that wheat embryo EF1, like bacterial EF Tu, interacts with aminoacyl-tRNA but not with unacylated tRNA in the presence of GTP^{6,7}. Figure 1*a* shows the elution of uninjected yeast ^3H -tRNA on a Sephadex G-200 column after incubation with EF1 and GTP. This preparation which has been chemically deacylated fails to interact with the protein factor and elutes as free tRNA (molecular weight 25,000). In Fig. 1*b* the same experiment is performed with yeast ^3H -tRNA that has been aminoacylated *in vitro* with a crude preparation of wheat aminoacyl-tRNA synthetases and the 20 different non-radioactive amino acids. It is clear that approximately 40% of the tRNA now elutes as a heavier peak (molecular weight 80,000) indicating that by aminoacylation it has acquired the capacity to interact with EF1 and GTP. In a separate experiment in which GTP was absent from the incubation mixture and the column buffer (not shown), the tRNA aminoacylated *in vitro* did not elute with a heavy peak but as in Fig. 1*a*. Figure 1*c* shows the results obtained with the original unacylated yeast ^3H -tRNA recovered 5 h after its injection into *Xenopus* oocytes and incubated with EF1 and GTP. Its elution pattern has changed drastically from what was observed in Fig. 1*a* since now 80% of the ^3H -tRNA elutes in the ternary complex fraction. To ascertain that this behaviour was due to *in vivo* aminoacylation, fractions 40 to 56 represented in Fig. 1*c* were pooled and chemically deacylated and, after concentration, run again with EF1 and GTP. Figure 1*d* shows that deacylation of the tRNA recovered after microinjection destroys its capacity to interact with EF1 and GTP.

It may be concluded therefore that the great majority of the yeast tRNA species microinjected as unfractionated tRNA can be aminoacylated very efficiently by the living oocyte. The amount of yeast tRNA introduced into each cell was approximately 100 ng which is 2.5 times the amount of endogenous tRNA⁸.

Direct quantification of *in vivo* aminoacylation in injected oocytes was attained by the use of a pure species of tRNA. The assay consisted in measuring the appearance of radioactivity precipitable with cold TCA in the aqueous phase after phenol extraction of oocytes that had been incubated in a medium containing ^{14}C -phenylalanine. Table 1 shows that no detectable radioactivity is obtained when uninjected oocytes or oocytes injected with unfractionated tRNA are treated. The amount of tRNA specific for phenylalanine in these cells is too low to be detected in the conditions used. Oocytes microinjected with purified tRNA^{Phe}, however, show considerable incorporation of radioactivity in the tRNA fraction. The identity of this radioactivity as aminoacyl-tRNA is established by its sensitivity to pancreatic RNase, mild alkaline hydrolysis and to hot TCA treatment. Incubation of the oocytes injected with tRNA^{Phe} with ^{14}C -threonine does not yield radioactivity bound to the tRNA. In the table it is also shown that microinjection of tRNA^{Phe} whose amino acid acceptor capacity

Table 1 The aminoacylation of pure yeast tRNA^{Phe} microinjected into oocytes

Experiment	Oocytes	Treatment	c.p.m. precipitated with cold TCA per oocyte
1	Uninjected		55
	Injected with 8 pmol of unfractionated yeast tRNA		88
	Injected with 8 pmol of pure yeast tRNA ^{Phe}		1,150
	Same as above	¹⁴ C-Thr instead of ¹⁴ C-Phe	42
2	Uninjected		38
	Injected with 8 pmol of tRNA ^{Phe}		840
	Same as above	RNase hydrolysis pH 10 for 15 min	72
	Same as above	90 °C for 15 min in 5% TCA	129
3	Uninjected		49
	Injected with 8 pmol of pure yeast tRNA ^{Phe}		57
	Injected with 16 pmol of yeast tRNA ^{Phe} oxidised		1,285
			43

The aminoacylation of tRNA^{Phe} was assayed by incubating duplicate groups of five oocytes each with ¹⁴C-phenylalanine of a specific activity 270 $\mu\text{Ci } \mu\text{mol}^{-1}$ at a concentration of 3×10^{-5} M for 2 h at 21 °C in 50 μl of a modified Holtfreter's medium containing 20 mM Tris-HCl pH 7.4 and 10 ng ml⁻¹ each of penicillin and streptomycin sulphate, 63 mM NaCl, 0.7 mM KCl, 0.9 mM CaCl₂, 1.0 mM MgCl₂ and 0.22 mM NaHCO₃. The oocytes were extracted by homogenising in 4 ml of cold mixture containing 2 ml of buffer (0.2 M sodium acetate 10 mM MgCl₂, 5 mM EDTA and 0.1% Triton X-100) and 2 ml phenol. The water phase was separated by centrifugation and duplicate aliquots of 0.5 ml were precipitated with cold 5% TCA. The precipitate was retained on Millipore membranes and the radioactivity was measured in a scintillation counter with 60% efficiency. Unfractionated yeast tRNA was purchased from Calbiochem; pure yeast tRNA^{Phe} from Boehringer. The yeast tRNA^{Phe} was oxidised¹¹ but the amine treatment not included. When ¹⁴C-threonine replaced the radioactive phenylalanine (experiment 1) the specific activity was 181 $\mu\text{Ci } \mu\text{mol}^{-1}$ and the concentration of 5×10^{-5} M. In experiment 2, where indicated, 0.5 ml aliquots of the water phase after phenol extraction were treated with 10 μg of pancreatic RNase or with mild alkali or heated in 5% trichloroacetic acid as given in Table 1.

has been destroyed by periodate oxidation fails to incorporate radioactivity.

Figure 2a shows the time course of the aminoacylation in oocytes injected with 20 pmol of tRNA^{Phe} per cell. The reaction is not yet complete after 2 h incubation when at least 25% of the injected tRNA has been aminoacylated with ¹⁴C-phenylalanine. Exact estimates of the amounts of Phe-tRNA synthesised would have to consider whether the radioactive amino acid is diluted by the internal pool of free amino acid in the oocyte before it is incorporated in the tRNA. The amount of free phenylalanine in *Xenopus* oocytes has been estimated to be approximately 60 pmol per oocyte (Bravo and Allende, unpublished) a quantity that would yield a fourfold dilution of the ¹⁴C-phenylalanine taken up by the cell in 2 h. Recent evidence obtained in other cells indicates that this dilution may not take place^{9,10}.

The effect of injecting different amounts of tRNA^{Phe} on Phe-tRNA formation in the oocytes can be seen in Fig. 2b. In this experiment the incubation was carried out for 2 h so that the values obtained with the lower tRNA^{Phe} concentrations represent extent rather than rate of aminoacylation. With limiting amounts of tRNA^{Phe} aminoacylation of 85–90% of the injected tRNA was obtained without considering dilution by the internal pool of free phenylalanine. This result is corroborative evidence that such dilution may not take place in this system.

Using the values shown in these figures it is possible to calculate that each oocyte has enough phenylalanine-tRNA synthetase to catalyse the aminoacylation of approximately

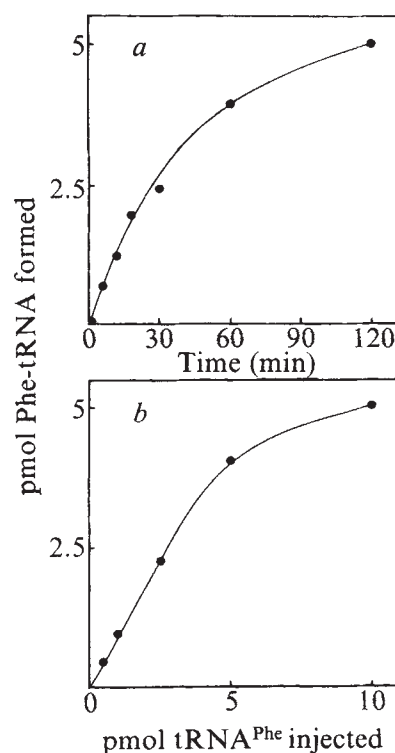


Fig. 2 The effects of time and tRNA concentrations on the *in vivo* aminoacylation of tRNA injected into *Xenopus laevis* oocytes. *a*, 20 pmol of pure yeast tRNA^{Phe} were injected into each oocyte and duplicate groups of five oocytes were incubated at 21 °C for the times indicated in amphibian saline medium containing 3×10^{-5} M ¹⁴C-phenylalanine, specific activity of 270 $\mu\text{Ci } \mu\text{mol}^{-1}$. The amount of aminoacylation was determined as described in Table 1 and the pmol of aminoacylated tRNA^{Phe} per oocyte were calculated disregarding cellular dilution of the specific activity of the ¹⁴C-phenylalanine. *b*, The amounts of tRNA^{Phe} indicated were injected into each oocyte of duplicate groups of five and incubated for 2 h in the same medium. The amounts of aminoacylation were determined as above.

10^{10} molecules of tRNA^{Phe} min⁻¹. This rate is approximately an order of magnitude higher than the rate of incorporation of phenylalanine into protein in these cells (Bravo and Allende, unpublished).

In conclusion, the results presented above indicate that tRNA microinjected into amphibian oocytes is functional in the sense that it can be aminoacylated *in vivo*. Cells microinjected with tRNA^{Phe} contain more than 500 times the normal concentration of this specific tRNA and 150 times the amount of Phe-tRNA. It should, therefore, be possible in the future to study the effects that such an unbalance provokes on protein synthesis and other cellular processes.

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Translation of natural mRNAs *in vitro* by extracts of a mutant in protein synthesis

NATURAL mRNAs contain specific signals for initiation and termination of protein synthesis^{1,2}. To further understand these processes and their regulation in the cell, we have been screening mutants defective in translation of natural mRNAs but not of artificial templates.

The temperature-sensitive *Escherichia coli* mutant, N4316, is one of this class. At 36 °C, *in vivo*, N4316 suppresses the termination codons UAA and UGA, but not UAG (ref. 3). At

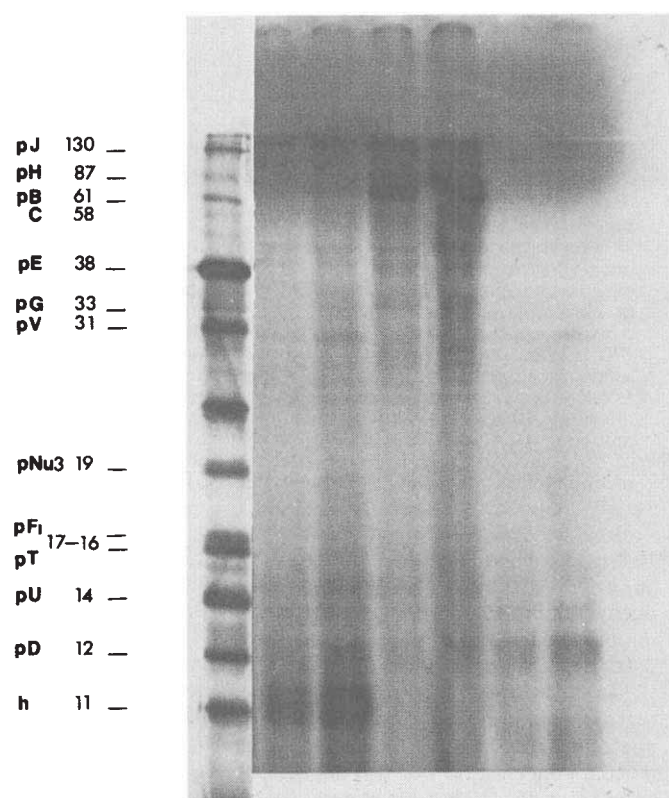


Fig. 1 Analysis of MS2 and MS2 (623) products on SDS polyacrylamide gels according to Maizel¹⁰ using 15% acrylamide and 0.8% bisacrylamide in the separating gel and 5% acrylamide in the stacking gel. Products were labelled with ³⁵S-methionine (see Table 2). Incubations were terminated with 13 mM EDTA, and 2 µg RNase I. After 8 min at 35 °C, proteins were concentrated by precipitation with 5 volumes of acetone at -20 °C. Electrophoresis was at 90 V for 1 h and 180 V until the tracking dye reached the end of the gels. X-ray films (RP X-Omat, Kodak Corp) were exposed for 4 d. Bacteriophage λ proteins labelled with ³⁵S-methionine were a gift from C. Epp and P. Ray (University of Toronto). Samples from left to right were as follows: λ proteins, 200 × 10³ c.p.m.; MS2, 20.4 × 10³ c.p.m.; MS2, 40.8 × 10³ c.p.m.; MS2 (623), 28.0 × 10³ c.p.m.; MS2 (623), 56.0 × 10³ c.p.m.; no RNA, 9.4 × 10³ c.p.m.; no RNA, 18.8 × 10³ c.p.m. Counting efficiency for ³⁵S was 50%. The leftmost channel contains phage λ late polypeptides labelled *in vivo* with ³⁵S-methionine. These are indexed according to their gene of origin; 'h' is a host peptide(s). The numbers refer to the molecular weights of these peptides in kdaltons. By extrapolation 'h' has a minimum molecular weight of 10,500. The major product of *in vitro* synthesis (coat protein) migrates like a peptide of molecular weight 10,800 ± 300. ¹²⁵I-labelled (or unlabelled) authentic coat protein¹¹ was found repeatedly to have the identical migration in these gels as the *in vitro* synthesised product.

Table 1 Protein synthesis by N4316 extracts programmed with amber mutants of the maturation and replicase cistrons of MS2 RNA

RNA	d.p.m. ³ H-histidine incorporated (× 10 ⁻³)		
	36 °C	46 °C	46 °C + RF
MS2	194	38.4	146
am5	41.0	23.5	66.0
am9	152	35.0	113

All experimental details were as described previously⁷ except that incubations contained 5 µCi ³H-histidine (58 Ci mmol⁻¹, Radiochemical Centre). Where indicated, 1.3 µg of rescue factor (RF) prepared without the DEAE cellulose step⁷ and 12.5 µg of each mRNA were used to programme the S30 extracts of N4316. Radioactivity incorporated into hot TCA-insoluble products was measured. *Am5* and *am9* lysates, gifts of Dr M. Kozak and D. Nathans (Johns Hopkins University) were grown as described⁹, and contained less than 0.1% revertants. Controls with extracts from the parental strain D₁₀, showed no temperature sensitivity and no stimulation by the rescue factor. Incorporation without mRNA, about 10% of the total, was subtracted from each value. Counting efficiency for ³H was 20%. Products were analysed as described by Ganoza *et al.*⁵. The bulk of the released MS2 and *am9* products correspond in molecular weight to maturation protein (40,000).

30 °C the mutant does not suppress³. This observation implied a temperature-dependent lesion in termination, and a moderate defect in this function was demonstrated *in vitro*⁴⁻⁶. The mutation, however, seems pleiotropic. At 43-45 °C, translation *in vivo* is impaired and cells die³. In extracts of N4316 synthesis of the coat protein coded by R17 and f2 phage RNAs is greatly reduced at restrictive temperatures⁴⁻⁶. The defect *in vitro* seems to be in an early function of translation⁷. This function does not involve the initiation reaction in the usual sense, since the rate and extent of formation of the Fmet-tRNA-ribosome-f2 RNA complex is unimpaired^{6,7}. The synthetic defect can be reversed by adding a soluble protein from wild type cells⁷. This "rescue" protein has been purified and is free of known enzymes and factors involved in protein synthesis⁷.

In this communication we further examine the temperature-sensitive defect in *in vitro* synthesis and its reversal by measuring systematically the reading of a number of cistrons from a spectrum of phage mRNAs. The measure of translation of specific cistrons was made possible through the use of mRNAs bearing known amber mutations. Our data indicate that the early translation lesion is general, and is the more drastic and important defect. Furthermore, the early defect in cistron reading is not the result of an interference with initiation by termination intermediates in the intragenic regions of the mRNAs.

To score for specific cistrons, histidine was used as label because it is not present in the coat protein and synthesis was programmed by early amber mutants of the maturation cistron (*am5*) or the replicase cistron (*am9*), so that only one protein was measured at a time. Protein synthesis in extracts of N4316 directed by *am9* is temperature sensitive and can be restored by rescue factor (Table 1). The *am9* mRNA directs synthesis and release of a product that corresponds in molecular weight to the maturation protein (unpublished).

Protein synthesis catalysed by N4316 extracts using the *am5* template is also temperature sensitive and the defect is reversed by rescue factor (Table 1). Analysis of the products on SDS polyacrylamide gels done after separation of nascent and free proteins, showed that the smaller level of translation with *am5* at 36 °C is due to a low level of replicase synthesis (unpublished). To overcome this problem, an amber mutant of the coat protein which overproduces replicase was used. As shown in Fig. 1, MS2 (623) RNA, programmes a product corresponding in molecular weight to the phage replicase. The synthesis of this product is temperature sensitive in N4316 and is reversed by the rescue factor (Table 2, experiment 1). Protein synthesis directed by Qβ RNA is also temperature sensitive and is restored by the rescue protein (Table 2, experiment 5).

These data show that the rescue protein works on all phage

cistrons and with all mRNAs tested (R17, MS2, f2 and Q β). The maturation protein is coded by the 5'-terminal segment of the phage RNA. Since synthesis of this protein is defective in extracts of N4316 (Table 1), it is unlikely that the impaired synthesis at restrictive temperatures is the result of a defect in the release mechanism.

Further evidence that the defect cannot be accounted for by a problem in termination only is given in Table 2. Synthesis programmed with a non-polar mutant (*aml1b*) of R17 phage is thermolabile and is reversed by the rescue protein. In *aml1b*, the UAG codon is 59 codons away from the normal signal for termination of coat protein synthesis. Synthesis programmed by *aml1* of f2 is also temperature sensitive (Table 2). This phage has two amber mutations, one in the maturation cistron, and the other at the 50th codon of the coat protein cistron. These experiments indicate that steric hindrance by ribosomes is not responsible for the impaired translation.

No coat protein is produced when the MS2 (623) RNA is used as messenger (Fig. 1). This rules out misreading of the amber codons by the N4316 strain. These experiments show that the mutant N4316 has a marked effect on translation

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Symmetry recognition hypothesis model for tRNA binding to aminoacyl-tRNA synthetase

THE fidelity of the transfer of the genetic information at the translational level depends largely on the accuracy with which each transfer RNA (tRNA) is aminoacylated by its cognate synthetase. The search for the common sites on tRNA which the enzyme recognises has resulted so far in a rather confusing picture. Practically all parts of the tRNA molecule have been implicated as the possible recognition site(s)^{1,2}.

Based on the general three-dimensional structure of tRNA (ref. 3) and the observation of the repeating sequences in various aminoacyl tRNA synthetases⁴⁻⁷, a model for the binding of tRNA and aminoacyl tRNA synthetase (aa-RS) is proposed where the internal symmetry of tRNA coincides with that between "unit domains" of the enzyme.

The three-dimensional structure of two crystal forms of yeast phenylalanine tRNA has been determined by the X-ray crystallographic method⁸⁻¹⁰. The structure is shown in Fig. 1. An examination of the structure reveals that the molecule contains two types of symmetry elements relating two regions: a pseudo two-fold axis and a pseudo twofold screw axis within a molecule as shown in Fig. 2. The regions related by the twofold axis overlap with, but are larger than, those related by the screw axis.

Some aa-RSs are composed of multiple subunits of the "smaller protomer" (molecular weight 35,000-50,000) and others contain one or more subunits of the "large protomer" (molecular weight 70,000-100,000)^{1,2}. It has been suggested that the "large protomer" is composed of two homologous sequences⁴⁻⁷. This sequence homology implies that the three-dimensional structure of the protomer is likely to contain two structural domains as was the case in the crystal structure of Fab' fragment of a human immunoglobulin¹¹, where the two structural domains in one chain are related to each other by a pseudo screw axis. A preliminary X-ray crystallographic study of *Escherichia coli* methionine tRNA synthetase, part of which was enzymatically cleaved off, shows a structural domain of a prolate ellipsoidal shape (Reisler, J.-L., unpublished) with approximate axial lengths 22 × 22 × 30 Å. The estimated molecular weight corresponding to the volume of this structural domain (based on the assumption that the partial specific volume of the average protein is 0.74 ml g⁻¹ (ref. 12)), 49,000, which is in the range of the expected value of 43,000 for one half of the protomer. The most likely symmetry elements relating two structural domains in such a protomer are a pseudo twofold axis and a pseudo screw axis. Thus, one such structural domain can be considered a "unit domain" and two or more such "unit domains", which are related by pseudo twofold and/or screw axes, could form the assumed three-dimensional structure of a functional enzyme.

The model proposed here is based on the following assumptions: First, the aa-RSs recognise the common tertiary structural features of tRNAs. This is supported by the observations that the misacylation occurs in heterologous systems. (for example, *E. coli* tRNAs charged by yeast enzymes^{13,14}) as well as in homologous systems¹⁴. Second, the specific recognition then selects the cognate mates. The hypothesis put forward is that the pseudo-symmetric regions of a tRNA are recognised

Table 2 Protein synthesis in extracts directed by different mRNAs

Experiment no.	RNA	d.p.m. incorporated ($\times 10^{-3}$)			
		40-41 °C	43 °C	43-46 °C	46 °C
1	MS2 (623)	229	259	62.0	110
2	R17	327	—	12.5	195
	<i>aml1b</i>	254	—	19.0	206
3	R17	181	—	18.5	152
	<i>aml1b</i>	217	—	16.5	176
4	f2	368	415	65.6	201
	<i>aml1</i>	329	328	50.4	197
5	Q β	761	—	35.5	345

Experimental details were as described (ref. 7 and Table 1) except that 5 μ Ci of ³H-lysine (3.7 Ci mmol⁻¹, New England Nuclear) as used in experiments 1, 2 and 5; 5 μ Ci ³⁵S-methionine (147 Ci mmol⁻¹, New England Nuclear) in experiment 4; and 5 μ Ci ³H-histidine in experiment 3. Where indicated 2.3 μ g of RF were used. Phages MS2 (623) (a gift from Gordon Carmichael, Harvard Biolabs) and Q β (a gift from David Hoar, University of Toronto) were grown and purified and the RNA extracted as described previously⁸. Revertants were less than 0.1%. Where shown, synthesis was programmed by 11 μ g of each RNA. Radioactivity incorporated into acid-insoluble products was measured. Optimum temperatures for synthesis were used as permissive temperatures as follows: experiments 1 and 4, 40 °C; and experiments 2, 3 and 5, 41 °C. Non-permissive temperatures were as follows: experiment 1, 43 °C; experiments 2, 3 and 4, 45 °C, and experiment 5, 46 °C. S30 extracts from N4316 were used in these experiments. Controls with the parental strain, D₁₀, showed no temperature sensitivity, and no stimulation by rescue factor with any of the RNAs. The predominant ³⁵S-labelled products directed by R17, f2 or Q β RNAs at 45 or 40 °C, with or without rescue factor, migrated on 10% acrylamide gels (ref. 10 and Fig. 1), with identical mobility to authentic coat protein extracted from f2 phage. Products smaller than coat protein were observed with MS2(623) or *aml1* RNA. Other bands observed after prolonged exposure with Q β or f2 RNA with and without factor were about 65,000 and 35,000-40,000 in molecular weight.

apart from its effect on chain termination. This defect affects translation of all mRNAs tested and all cistrons examined to approximately the same extent.

Other mutants of the same class exhibit similar phenotypes (unpublished). We therefore suggest that there exist functions vital to translation or its regulation in the cell which are not scored by any of the known partial assays of translation.

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by the pseudo-symmetric regions of an aa-RS consisting of more than one "unit domain". For the enzymes with two identical small protomers, the true symmetry is assumed to convert to the pseudo symmetry by an asymmetric conformational change either on dimerisation or on ligand binding. This "symmetry recognition" can be achieved by coinciding the symmetry axes of the same type from tRNA and the enzyme, for example, the pseudo twofold axis of the tRNA with the pseudo twofold axis of the enzyme, while maximising the contact area as shown schematically in Fig. 3. In making the schematic complex, the regions of nucleotide sequence which vary the most among different tRNAs (dotted portion in Fig. 1) are kept further away from the enzyme and the overall contact area on tRNA is localised more on the "inside or diagonal side" of the L-shaped tRNA as has also been suggested by Rich and Schimmel (unpublished).

The symmetry-matching process will align tRNA and the enzyme in register and provide contact areas including some

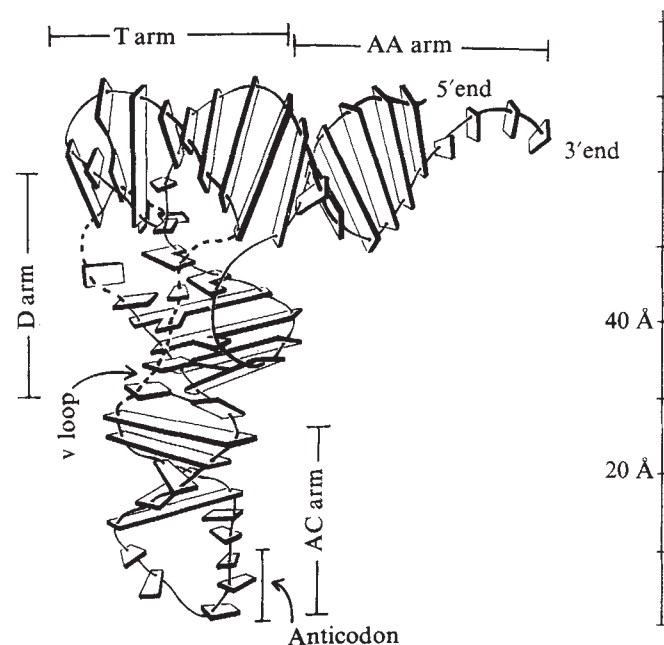


Fig. 1 Schematic drawing of the 3-D structure of yeast phenylalanine tRNA in the orthorhombic crystal⁹. The amino acid acceptor (AA) arm and the T ψ C (T) arm form a continuous double helix and the anticodon (AC) arm and the DHU (D) arm form the other partially continuous double helix. These two helical columns form an "L", where the D and T loops are at the corner of the "L". The phosphate-ribose backbone is shown as a continuous wire except for the three dotted segments, variable (V) loop and two segments in the D loop. The number of nucleotides in these three segments varies among different tRNAs. The secondary and tertiary base pairs are shown as long or bent slabs and the additional tertiary hydrogen bonds between the bases are shown as dark straight lines. Tertiary hydrogen bonds involving riboses and phosphates are not shown. The molecule is rather flat with the approximate thickness of 20 Å. The backbone conformation at the 3'-end in the crystal is more extended than shown here.

non-symmetric regions. The contact areas on the enzyme are not expected to be one continuous elongated crevice, but two partially isolated regions, one on each "unit domain". Similarly, the contact areas on tRNA are expected to be centred around parts of two symmetric regions and their neighbourhoods. There are two alternative general contact areas on tRNA as shown in Fig. 3c and d, both of which include two symmetry-related regions (AA and AC stems) as well as parts of the AC loop and the D stem (for abbreviations, see the legend of Fig. 1). Most of the implicated enzyme recognition sites (AA stem^{1,2,15}, AC stem¹⁶, D stem¹³, anticodon^{1,2,17}) belong in the symmetric regions or their neighbourhoods. More of these implicated recognition sites are in contact with the enzyme in the model shown in Fig. 3c than the one in 3d.

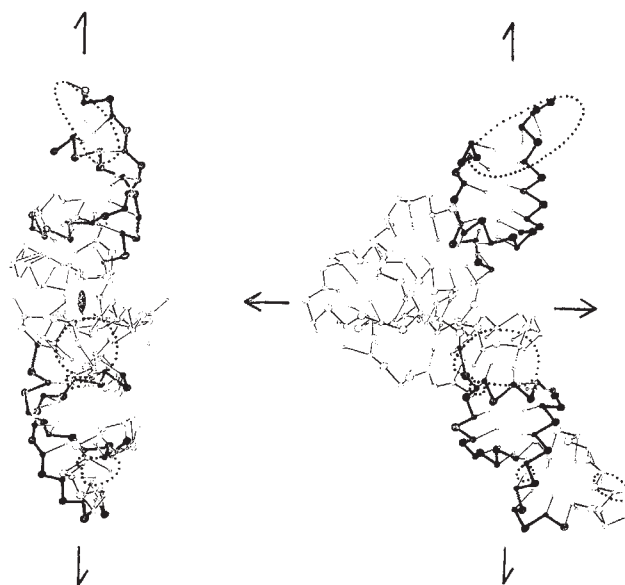
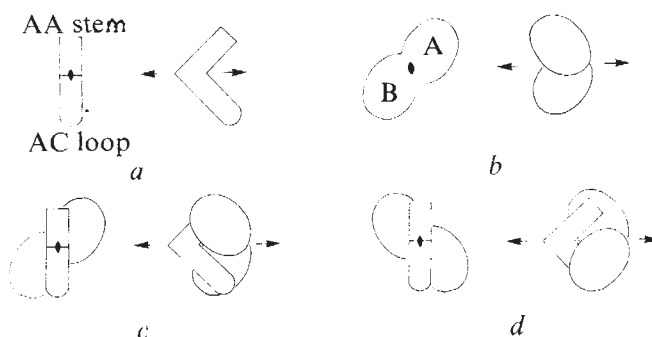


Fig. 2 Two views of yeast phenylalanine tRNA in the orthorhombic crystal⁹. The large, medium and small circles represent the centres of the phosphates, the riboses, and the bases, respectively. The pseudo twofold axes are indicated by arrows when on the plane and by a solid ellipse when perpendicular to the page. The pseudo twofold screw axes are indicated by one-headed arrows. The twofold symmetry related backbones are shown in black. The backbones related by twofold screw axes are shorter than those related by twofold axes. A few nucleotides at the 3'-terminal end are not shown. The aa-RS recognition sites implicated by various experiments^{1,2,13,15-17} are circled by dotted lines.

The specific recognition sites of tRNA for the cognate enzymes are likely to be different for different tRNAs within the contact areas, but the common scheme by which tRNA and the enzyme align is proposed to be by the recognition of the pseudo-symmetric regions on both molecules.

The model proposed here enables one to make a few predictions. First, any increase or decrease in the symmetry in the pseudo symmetric regions (AA and AC stems primarily) will lower tRNA-enzyme binding properties. Such change of the symmetry can occur when a base pair is replaced by an unusual base pair such as a GU pair, or vice versa. The charging specificity may or may not change because the specific recognition site(s) may be in or in the neighbourhood of the symmetric regions. Second, any alterations in the T arm or D loop (and V loop in case of the model shown in Fig. 3c) which do not change the relative positions of the AA and AC stems and the pseudo-symmetry between them will not affect the binding or charging

Fig. 3 Schematic drawings showing the matching of the pseudo twofold symmetry axes of tRNA and aa-RS: two orthogonal views of a, tRNA; b, two symmetry related "unit domains" in an aa-RS; and c and d, two alternative ways of forming tRNA-enzyme complex models by coinciding the twofold axes of both molecules. The matching of the pseudo screw axes can be achieved in a similar fashion with slightly different arrangement between the two molecules.



specificity. Third, for each model complex (Fig. 3c or d) there are two ways tRNA can interact with the enzyme: one is as in Fig. 3c or d, the other is by keeping two "unit domains" the same but rotating tRNA by 180° around the pseudo twofold axis, allowing the AC stem to interact with "unit domain" A and the AA stem to interact with B (see Fig. 3b). Since the symmetry is not true, these two modes of binding are likely to show two different tRNA binding properties.

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Influence of conformation on effect of macroions on kinetics of ionic reactions

POLYELECTROLYTES are known to alter drastically the rate of many ionic reactions in solution^{1,2}. In general, it may be said that the polyelectrolyte effect has an important role in the observed changes of the rates of reaction. The high electrostatic field in the neighbourhood of the macroion generates an inhomogeneous distribution of the ionic reactants—counterions being concentrated in a rather small volume surrounding the polymeric chains. It has been clearly shown^{3,4} that even when the ionic substrate is decomposed by specifically reactive groups incorporated into the polyelectrolyte molecule, the electrostatic interaction is the causative factor of the observed accelerated rate of decomposition. This polyelectrolyte effect may be a relevant factor on the activity of catalytic biopolyelectrolytes, for example enzymes.

So far, almost all the polymers studied have had a random coil conformation. Since conformation is a very important parameter in determining the catalytic activity of natural biopolyelectrolytes, we compare here the accelerating power of the α -helix conformer of a poly(amino acid) in solution to that observed in the presence of the corresponding random coil conformer.

Previously, the effect of the conformation of poly(L-glutamic acid) in solutions on the rate of polymerisation of vinylpyridine has been reported⁵. Rates were found higher for the α -helix conformer, but the mechanism and interpretation of the effect is not straightforward. Overberger and Podsiadly⁶ have demonstrated the importance of polymeric chain aggregation on the rate of a solvolytic reaction. This conformation involves only intermolecular hydrogen bonding, consequently it has no bearing on intramolecular hydrogen bonding occurring in biopolymers.

The rate of decomposition of 2,4-dinitrophenylphosphate

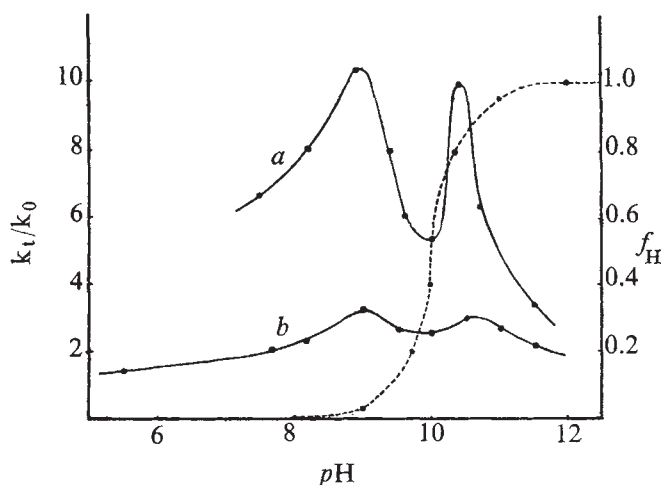


Fig. 1 Acceleration factor k_t/k_0 ($k_0 = 0.19 \times 10^{-4} \text{ s}^{-1}$) for the decomposition of DNPP against pH in the presence of poly(L-lysine). *a*, $R = [\text{poly(L-lysine)}]/[\text{DNPP}] = 290$; *b*, $R = 100$. Dashed line, fraction of α -helix, f_H , of poly(L-lysine) against pH (refs 7 and 10). ●, present work. The substrate decomposition was followed spectrophotometrically at 358 nm. At this wavelength the two reaction products, dinitrophenol and dinitro-(substituted) aniline, absorb in alkaline solutions. Alternatively, the formation of the aniline alone may be followed in acidic medium ($\text{pH} < 3$) at the same wavelength^{3,8,9}. All the rate measurements were performed at $30 \pm 0.1^\circ \text{C}$, and the course of the reaction followed up to 50% conversion; in this range the kinetic law is strictly first order for DNPP.

(DNPP) in solutions of poly(L-lysine) (PLL), a poly(amino acid) capable of undergoing a pH-sensitive α helix $\rightleftharpoons$ random coil conformational transition⁷, was determined. DNPP reacts specifically with the free- NH_2 groups in simple amine solutions^{8,9} as well as in poly(ethyleneimine) solutions (E.B., R.F.-P., and D.T., unpublished), in an analogous fashion to *p*-nitrophenyl phosphate³. The same reaction takes place with the free- NH_2 groups in PLL. The DNPP decomposition occurs by way of the dianion ($\text{p}K_2 = 4.5$) throughout all the pH range studied. In this pH region the hydrolysis of DNPP in water is independent of pH (refs 8 and 9 and E.B., R.F.-P., and D.T., unpublished).

The conformational transition of PLL in the presence and absence of the substrate was followed with circular dichroism measurements (JASCO J-20 Recording Spectropolarimeter). As shown in Fig. 1, the transition is not affected by the presence of the substrate and the values agree with those reported in the literature^{7,10}.

Figure 1 shows the results of the kinetic experiments at various pH. The acceleration of the decomposition is expressed as k_t/k_0 , k_t being the first-order rate constant in poly(amino acid) solutions at each pH value. The rate constant k_0 is the corresponding value in water since at the concentrations used in this work simple amines do not contribute significantly to the rate of decomposition of DNPP^{3,8,9}. Figure 2 shows the pH profile of k_t/k_0 for DNPP decomposition in solutions of poly(DL-lysine) (PDLL), which does not undergo the α helix $\rightleftharpoons$ coil transition; at all pH values it exists as a random coil¹¹. The maximum at pH 8.9 in PLL solutions (Fig. 1) and that observed in PDLL solutions (Fig. 2) correspond to the effect of the random coil polyions on the rate of DNPP decomposition. A single maximum has also been observed for the decomposition of this substrate and *p*-nitrophenyl phosphate in solutions of random coil macroions possessing $-\text{NH}_2$ groups in their molecules (ref. 3 and E.B., R.F.-P., and D.T., unpublished). At the pH of the first maximum in Fig. 1, PLL is 98% random coil. These maxima are accounted for by the decreasing charge density of the weak polyelectrolyte, and by the increasing number of free $-\text{NH}_2$ groups when the pH of the solution increases (which may be considered similar to bifunctional catalysis). Further support for this interpretation

is given by the data illustrated in Fig. 3. It may be seen that k_t/k_0 for PLL random coil increases up to a constant maximum value as the concentration ratio $R = [\text{PLL}]/[\text{substrate}]$ increases. The constant k_t/k_0 value is attained when all the dianion is concentrated close to the chain where the reaction with $-\text{NH}_2$ groups takes place. The minimum value of R at which k_t/k_0 becomes constant is dependent on the charge of the substrate and on the charge density on the macroion, as demonstrated in previous work^{3,4}.

The model postulates two stages for the DNPP reaction: first, attraction of the substrate dianion to the vicinity of the polymeric chain, and second, attack of DNPP ions by the free- NH_2 groups on the macroion. Any change of charge density or number of free amino groups must give rise to changes in k_t/k_0 . One way of producing these changes is to alter the pH, as shown in Fig. 2 and the first maximum in Fig. 1. Another way is to alter the conformation of PLL, the transition taking place at pH 10.0. Figure 1 shows that a second maximum appears at pH 10.4 in the α -helix region (82% of PLL is in the α -helix conformation at pH 10.4). In spite of the smaller degree of ionisation of PLL in the alkaline solutions, where the second maximum is observed, the fact that the average distance between adjacent lateral groups, measured along the polymeric chain, is reduced from 3.6 Å, for the random coil, to 1.5 Å for the α helix¹¹, produces a marked increase in k_t/k_0 . Thus, a new maximum appears in the accelerating factor due to the sudden increase in charge density of PLL and possibly also, in the availability (or increase in local concentration) of $-\text{NH}_2$ groups in the α -helix conformer. The magnitude of the factor by which the α helix is more effective than the random coil in accelerating the rate of DNPP decomposition was estimated using the equation,

$$(k_{\text{helix}} - k_0)/(k_{\text{coil}} - k_0)$$

The rate constants k_{helix} and k_{coil} for the pure conformers were calculated from the observed k_t and the fraction of α helix (f_H). The comparison was made with data corresponding to pH 10.4, the α helix being 5.5 times more effective than the random coil. If the data refer to a degree of ionisation of 0.11 for the amino groups of both conformers, the α helix becomes 12 times more effective.

The proposed model is so far only qualitative and it is

Fig. 2 k_t/k_0 against pH for the decomposition of DNPP in the presence of poly(DL-lysine) ($R = 400$).

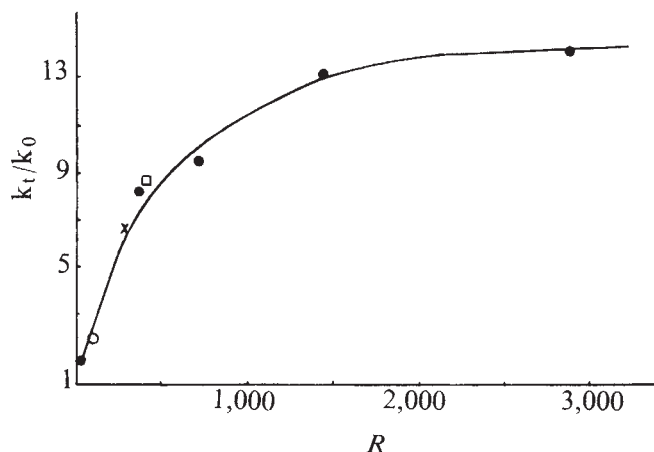
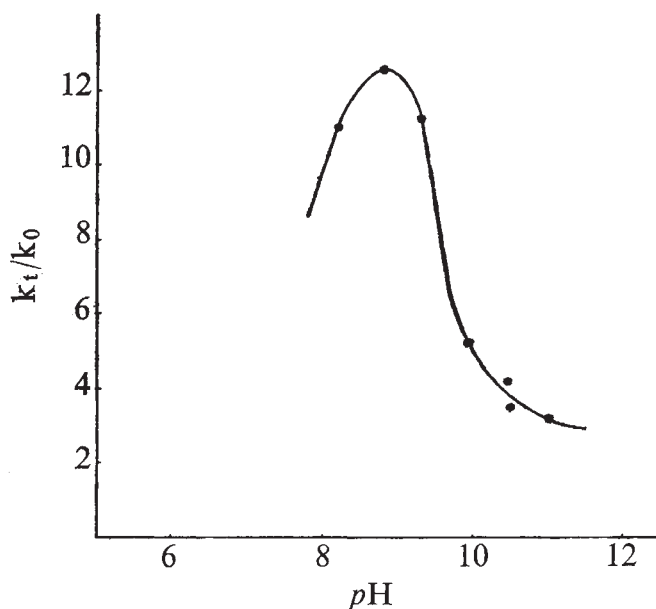


Fig. 3 k_t/k_0 against R , measured at degree of neutralisation, $i = 0.9$. ●, $[\text{DNPP}] = 6.7 \times 10^{-6} \text{ M}$, plus the corresponding amounts of PLL; ×, $[\text{DNPP}] = 8.3 \times 10^{-6} \text{ M}$, $[\text{PLL}] = 2.4 \times 10^{-3} \text{ N}$; ○, $[\text{DNPP}] = 2.4 \times 10^{-5} \text{ M}$, $[\text{PLL}] = 2.4 \times 10^{-3} \text{ N}$; □, $[\text{DNPP}] = 8.3 \times 10^{-6} \text{ M}$, $[\text{PLDL}] = 3.3 \times 10^{-3} \text{ N}$.

probable that other interactions (hydrogen bonding, local environment and so on) also contribute to some extent.

We thank Professor A. Paladini for the CD measurements and the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) for financial help.

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Errata

In the article "Voltage distribution across nerve membranes", by M. Sternberg, I. Lundström and L. Lundkvist (*Nature*, **255**, 496; 1975) the first line of equation (1) should read $V_a = (V - V_0)/[1 - (2\epsilon_a \sigma^2 / \epsilon^2 a d \cdot K)]$.

In the article "Helper function of T7 protein kinase in virus propagation", by M. Hirsch-Kauffman, P. Herrlich, H. Ponta and M. Schweiger (*Nature*, **255**, 508; 1975) the labelling of *E. coli* in both the figure and legend should be: a, *E. coli* B_s-1; b, *E. coli* K12 514.

reviews

THE current generation of physicists and engineers is probably more familiar than any of its predecessors with the name of Fourier because of the many modern applications of Fourier analysis and Fourier transforms, which range from diffraction theory and spectroscopy to circuit analysis and quantum mechanics. In the 19th century Fourier profoundly influenced Kelvin and Heaviside; and as early as 1833 William Rowan Hamilton was writing of "Fourier, whom I place at the head of the French School of Mathematical Philosophy, even above Lagrange and Laplace".

So the prospective reader of a biography of Fourier* might expect to find an account of a typically great theorist, probably withdrawn from the buffetings of normal life, in accordance with the popular picture of a man of science. But that picture is open to question, especially as regards those generations of French scientists of which Fourier was a member. Maupertuis fought in the army of Frederick the Great. Lazare Carnot was the "organiser of victory" and the planner of Napoleon's most successful campaign. Charles, of Charles' Law, was one of the crew of two in the first manned flight of a hydrogen balloon. And Gay Lussac for many years held the world's balloon altitude record of 23,000 feet, which he established in 1804. But as is well brought out in Dr Herivel's new biography, none of these experiences was more intense than those of Fourier, whose life combined science and action in a remarkable way. When he started his classical work on the theory of thermal conduction he was about 35 years old—a distinctly late beginning

for a theorist—and he had already had a most adventurous career.

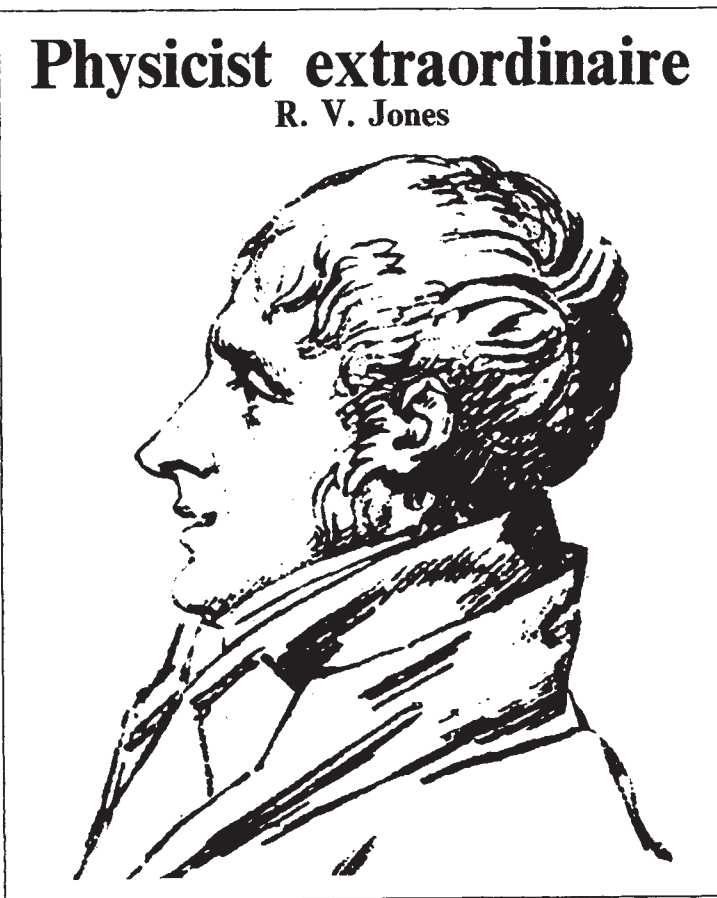
Born at Auxerre in 1768, he went to the Ecole Militaire in that town, and thence to the Abbey at St. Benoit with the intention of entering the Benedictine Order. Returning to Auxerre without taking his vows, he was made an Abbé in 1790; but he became so caught up in the maelstrom of the French Revolution as to be named at one time for execution; and a little later he became President of the Revo-

this Expedition, Fourier was held as hostage for all the other savants by Admiral Sir Sidney Smith, an experience which resulted in friendship rather than resentment, for the Admiral saved Fourier's papers from destruction and later returned them to him in France.

Fourier became Prefect of the Department of Isère and was stationed at Grenoble, where he succeeded in organising the draining of the swamps of Bourgoin, a project which had been afoot since the time of Louis XIV. At the same time he was editing the general introduction to the *Description of Egypt*, the great compilation of the results of the Egyptian Expedition. Created a Baron in 1802, he was still Prefect of Isère in 1815—and still at Grenoble, right in the path of Napoleon's famous march on Paris. Vacillating about the surrender of Grenoble to his old Emperor, who had to batter the gates open after Fourier had conveniently withdrawn himself to the country, he incurred Napoleon's displeasure and was dismissed from his post as Prefect, only to be appointed Prefect of the Rhône by a mollified Napoleon a few days later. But after Waterloo, Fourier's Napoleonic favours did not endear him to Louis XVIII, and so within three months he was removed from his second prefecture. Louis indeed refused for a time to confirm Fourier's election to the Académie des

Physicist extraordinaire

R. V. Jones



lutionary Committee in Auxerre and, as such, a chief agent of the Terror. As so often happened, this republican prominence rebounded, and he himself was imprisoned in 1794. Released, he was nominated to the Ecole Normale, only to be imprisoned once more in Paris in 1795. Again released he went to the École Polytechnique, where in 1797 he succeeded Lagrange in one of the Chairs. In 1798 he was summoned to join Napoleon's Expedition to Egypt, along with many other eminent men of learning. After the military defeat of

Sciences but relented in 1817, and from 1822 until his death in 1830 Fourier was permanent Secretary of the Académie. All these events are described with great historical care by Dr Herivel, who has provided an illuminating selection of Fourier's letters, along with many helpful biographical notes about the multitude of characters with whom Fourier became involved.

It was during his period in Grenoble that Fourier worked on the conduction of heat; and, as Dr Herivel emphasises, his first basic contribution was to

**Joseph Fourier: The Man and the Physicist*. By John Herivel. Pp. xi+350 +5 plates. (Oxford University Press: London; Clarendon: Oxford, April 1975.) £9.75.

postulate that the flow of heat could be treated as that of an indestructible substance. In view of its antecedence in the caloric theory, and of its subsequent universal acceptance, it is hard to appreciate that Fourier had great difficulty in persuading Laplace, Biot and Poisson of its validity. Their doubts about his physics were reinforced by parallel doubts about his non-rigorous treatment of trigonometrical series, but in both instances his instincts proved correct. Refusing to be discouraged by the objections, Fourier developed his treatment of the heat conduction problem into one of the classical triumphs of physics, and incidentally developed his use of trigonometrical series in the solution of his differential equations of conduction. All that is well brought out by Dr Herivel, who has deliberately concentrated on Fourier as a physicist and a man, on the grounds that Fourier the mathematician has been well covered. But I would have been grateful had some of Fourier's mathematics been included in the same volume because although, even if we agree with Dr Herivel that "It would be unrealistic to strike a balance between Fourier the man and Fourier the savant", the interplay of mathematics

and physics in Fourier's achievements must be a phenomenon of great interest. But, even regarding Fourier as a physicist alone, it is hard to understand why there is no reference in the book to Fourier's invention of the method of dimensions, which almost every subsequent physicist has used (Maxwell, for example) and which is a sure criterion of Fourier's insight.

Dr Herivel almost conveys a sense of disappointment that conspicuous though Fourier's civic role was it "can merit no more than a footnote in the history of the period whereas both as a physicist and as a mathematician he was undoubtedly one of the major figures of the nineteenth century and beyond". But what is really remarkable, as in the case of Handel, is that interspersed among the passages of a tempestuous and worldly career, Fourier should have achieved such heights of contemplative creation. Men of science who still have to battle with administrators who are reluctant to regard them as equals may point with gratitude to the example of Fourier. And their gratitude may be extended to Dr Herivel for this, within its limits, excellently annotated and documented account of Fourier and his times.

Tissue connection

Connective Tissue: Macromolecular Structure and Evolution. (Molecular Biology—Biochemistry and Biophysics, vol. 19.) By Martin B. Mathews. Pp. xii+318. (Springer: Berlin and New York; Chapman and Hall: London; May 1975.) £15.30.

THIS monograph, the 19th in the series *Molecular Biology—Biochemistry and Biophysics*, is an excellent review of the macromolecular structure and evolution of connective tissue. During recent years the advances in this field have been substantial indeed and this is the first time that an attempt has been made to consider this vast, widely scattered literature in the context of evolution and taxonomy.

The general construction and arrangement of the book are admirable. The first six chapters, amounting to about half the text, consist of a concise survey of the biochemistry of the macromolecular constituents of connective tissue: collagen, elastin, structural glycoproteins and polyanionic proteoglycans. The emphasis lies very much on the description of primary levels of organisation, although relevant conformational data are mentioned and occasionally described. Each chapter also presents a most comprehensive account of the comparative biochemistry of these macromolecules, leading to considerations of molecular phylogeny. These considerations are,

however, hampered by the rather limited amount of structural information, particularly concerning amino acid sequences, which is now available. The coverage of collagen and glycosaminoglycans is extensive and this makes even more noticeable the somewhat limited space given to the discussion of elastin. This is unfortunate as the structure of that protein must still be regarded as controversial and the reader would have been helped by a more critical appraisal of the literature.

Professor Mathews' own particular interests—changes in cartilage biochemistry with embryonic development, maturation and ageing, and the taxonomic distribution of glycosaminoglycans—are presented in the next three chapters. These contain separate sections for individual tissues, among which particular emphasis is given to cartilage, bone and notochord. Apart from their evolutionary significance, they are a mine of information and contain some original, unpublished work from the author's laboratory.

The last chapter is concerned mainly with the molecular properties and interactions of collagen and proteoglycans and because it encompasses so many facets of the subject, it should appeal to readers from many other disciplines.

The style is direct and unpretentious and the author admirably combines erudition and lucidity of exposition. This book will be most welcome to those working in this field.

A. Serafini-Fracassini

Environmental mechanics and thermodynamics

Fluid Mechanics and Thermodynamics of our Environment. By S. Eskinazi. Pp. xv+422. (Academic: New York and London, February 1975.) \$26.00; £12.50.

IN comparison with other books on the motion of fluids by the same author, this one is a little disappointing in its production and layout. According to the preface the word 'environment' takes a variety of meanings and it is a word which is certainly made use of far too often in the text; usually, the more precise term 'atmosphere' or 'ocean' would suffice in its stead. The book is aimed at engineering students interested in air-borne and water-borne pollution but, though there is an obvious need for a concise introductory text which covers this field, this presentation leaves one with the impression that it is partly an attempt to jump on the current bandwagon of concern over man's use of the Earth as a waste disposal unit. The diagrams are the worst feature of the book and are sometimes amateurish in style. Their reproduction is usually on so small a scale that details tend to be obscured.

On the good side, the book is a useful introduction to both physical meteorology and oceanography, necessary parts of the curriculum of anyone studying pollution. Usually, these two subjects are treated separately but here the author has brought together their simpler aspects in one unified text and has attempted to present the material in a manner not requiring formal courses in fluid mechanics and thermodynamics as prerequisites. On the whole he has succeeded although a good background in physics and mathematics is essential for a full understanding of the content of the book. Nevertheless, in one or two places, the reader is plunged into discussions of turbulent flows without the benefit of earlier treatment of simpler laminar models. Although it can be argued that flows in the atmosphere are rarely laminar, the difficulties of the treatment of the motions of a real atmosphere are often daunting to an undergraduate with no training in hydrodynamics.

The book should prove most useful to the experienced scientist or engineer who requires a knowledge of the rudiments of meteorology and oceanography. It may also find some application in courses in geophysics which are not solely confined to a study of the solid Earth.

C. W. Titman

Non-earth science

Planetary Geology. By Nicholas M. Short. Pp. xv+361. (Prentice-Hall: Englewood Cliffs, New Jersey, April 1975.) \$17.95.

THE content of this book is both narrower and wider than the title *Planetary Geology* would imply. The only extraterrestrial body to have been visited by man, extensively sampled and studied with a variety of on-site instruments is, of course, the Moon; and so it is hardly surprising that over 50% of the book should be devoted to lunar studies. Making allowances for the general introduction, the summing up and a chapter each on meteorites, the origin of planets and general aspects of the Solar System, only 15% of the book remains for "Mars, Venus, and the planets beyond"—a fair reflection of the present state of knowledge.

On the other hand, the term 'geology' is plainly inadequate, even for the seven chapters which summarise our physico-geological knowledge of the Moon. Dr Short is an employee of NASA and has used the perspective this gives him to write a book which sets the science firmly against the operational and methodological background

of lunar exploration. This "inquiry-and problem-oriented" approach gives the book a distinctive flavour which I found a welcome change from the artificial world of the average text. The inclusion of wider contextual details may lead to some cavilling among academic purists, but the effect is surely to enhance rather than detract from the purely scientific story.

In a fast-moving field such as this, rapid dating of material is an obvious and serious problem. This book takes account of work carried out by to July 1974 (a remarkable achievement under present circumstances), which makes it the most up-to-date and comprehensive text on the subject now available. It would be a pity if it were allowed to age, for it forms an excellent foundation upon which to build later results; and I hope that Dr Short will be granted both the facilities and inspiration for frequent updating. Not that he is a stranger to revision. The first draft of this text was used and evaluated by students and then extensively rewritten in the light of the criticism and suggestions received. This is a procedure which many other authors could well adopt with benefit to themselves and advantage to their suffering readers.

Peter J. Smith

and not histones, might contain regulatory elements.

These proteins are complex. Upwards of 50 individual peptides (not all of which are strictly acidic) can be demonstrated and many, perhaps most, are probably not involved in regulation. Like a gold strike, this area is, therefore, full of promise, challenge and excitement; but for every worthwhile discovery, there are many disappointments. Monographs on the subject, for this reason, have been slow to appear and this is one of the first to be devoted entirely to the acidic proteins of the nucleus. It is, therefore, of special timeliness and interest.

It has 10 contributions. Vincent Allfrey first discusses control in prokaryotic and eukaryotic systems, drawing analogies between them; he also outlines preliminary experiments on the isolation of specific DNA-binding proteins. Gordhan Patel then presents a detailed review of methods for the isolation and fractionation of acidic proteins, which is followed by an account by LeSturgeon and Wray of their work with phenol-soluble acidic proteins. The fourth chapter, by Lewis Kleinsmith, discusses modification by phosphorylation and the possible role of this in modulating nuclear function. Chapters 5 by Bruce McGunn and 6 by LeSturgeon, Totten and Forer are concerned mainly with the nucleoproteins of *Physarum polycephalum*. Chapter 7, by Berendes and Helmsing deals with the non-histone proteins of Dipteran polytene nuclei and is of particular interest since it brings together some biochemical findings and the morphological evidence about gene activity. The chapter, by the editors themselves reviews changes which have been reported in acidic proteins during cell growth. Tom Spelsberg devotes a chapter to a consideration of the role of nuclear acidic proteins in binding steroid hormones and, finally, Stewart Gilmour reviews the evidence that acidic proteins are involved in gene regulation in eukaryotes and discusses some of the models which have been proposed.

It is inevitable that any contemporary account of this field should be diffuse but gene regulation in eukaryotes is one of the greatest remaining challenges to the biologist and the acidic proteins of the cell nucleus are clearly implicated in it. The editors (Ivan Cameron and James Jeter) have managed to obtain contributions from several of the most experienced people in the field and the volume provides a reasonably comprehensive, up-to-date summary. It can, therefore, be recommended to laboratories in which work of this kind is being undertaken or contemplated.

John Paul

IAEA Publications

Thermodynamics of Nuclear Materials 1974

Volume 1

Proceedings of a Symposium, Vienna, 21-25 October 1974 £12 (£12.37)

Formation of Uranium Ore Deposits

Proceedings of a Symposium, Athens, 6-10 May 1974 £16.40 (£16.86)

Atoms, Molecules and Lasers

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Chromosomin re-emerges from limbo

Acidic Proteins of the Nucleus. (Cell Biology: A Series of Monographs.) Edited by Ivan L. Cameron and James R. Jeter. (Academic: New York and London, December 1974.) \$28.50; £13.70.

CHROMOSOMIN was described by Edgar and Ellen Stedman about 35 years ago as one of the three major components of chromosomes, the other two being nucleic acids and histones. They postulated that it could have a structural role in the chromosome whereas the histones were gene regulators. Partly because of this speculation and partly because chromosomin proved very refractory to handle, it disappeared into the limbo of half-forgotten things for a quarter of a century (during which time the histones were studied intensively) but re-emerged about 10 years ago, renamed the non-histone or acidic chromosomal proteins, as a result of experiments which suggested that they,

announcements

Awards

The 1975 **Hewlett-Packard-Europhysics Prize** for solid state physics has been awarded to **V. S. Bagaev, L. V. Keldysh, J. E. Pokrovsky and M. Voos.**

H. S. Schwartz has been awarded the **Johananoff International Fellowship** for advanced biomedical studies.

The Pontifical Academy of Sciences has awarded the **Pius XI Gold Medal** to **S. W. Hawkins** for his work on membranes.

Miscellaneous

Lunar Research. NASA is continuing to encourage and support research on all aspects of lunar science. Scientists with new ideas, techniques and special capabilities are encouraged to participate. NASA's lunar programmes include: experimental and theoretical research on lunar materials, lunar data analysis and synthesis, and supporting research and technology.

Further information from Dr E. A. Flinn, Director, Lunar Programs Office, Code SM, NASA, Washington, DC 20546.

International meetings

September 2-6, **Function of living plant collections in conservation**, Kew (Christine Brighton, Royal Botanic Gardens, Kew, Richmond, Surrey, UK).

September 9-11, **Applications and economics of remote sensing systems**, Silsoe, Bedfordshire (Mr T. S. Bell, ADAS, MAFF, Block B, Government Building, Brooklands Avenue, Cambridge, CB2 2DR, UK).

October 22, **Applications of analytical techniques to industrial effluents**, Colchester (Analytical Division, Chemical Society, 9 Savile Row, London W1X 1AF, UK).

October 26-November 1, **Alcoholism and drug dependence**, Sao Paulo, Brazil (International Council on Alcohol and Addictions, Box 140, 1001, Lausanne, Switzerland).

Reports and publications

Great Britain

- Potato Marketing Board. Sutton Bridge Experimental Station Annual Review 1974. 71. Summary of Investigations Undertaken in 1973/1974. Pp. 7. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) *gratis*. [106]
- Half the Loaf: A Study on the World Food Crisis. By Colin Pritchard. Pp. 41. (Edinburgh: The Saint Andrew Press, 1975.) 30p net. [116]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 270, No. 908: Excitable Membranes. A Discussion organized by R. D. Keynes, FRS. Pp. 295-559. (London: The Royal Society, 1975.) UK £10.70; Overseas £11.20. [116]
- The Forestry Commission's Objectives. Pp. 16. (Edinburgh: Forestry Commission, 231 Corstorphine Road, 1975.) [126]
- University of Bristol: Department of Agriculture and Horticulture. Report of the Long Ashton Research Station (The National Fruit and Cider Institute), for 1974. Pp. xxii + 220 + 7 plates. £2. An Introduction to Modern Cider Apple Production. By R. R. Williams. Pp. 34. 50p. (Long Ashton, Bristol: Long Ashton Research Station, The University, 1975.) [136]
- Report of the Tropical Products Institute, 1972/1974. Director: P. C. Spensley. Edited by Melba Kershaw and J. B. Davis. Pp. v + 96 + 12 photographs. (London: Tropical Products Institute, 127 Clerkenwell Road, EC1, 1975.) 90p net. [136]
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